

**A COMPARISON
OF THE
MECHANISMS OF ACTION
OF
CARVEDILOL AND METOPROLOL
IN
CHRONIC HEART FAILURE**

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Of science and the human heart
There is no limit
There is no failure here sweetheart
Just when you quit...

“Miracle Drug”, U2

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ABBREVIATIONS USED

ABTS	Azinodiethyl-benxthiazoline sulphate
ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitor
AF	Atrial fibrillation
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALLHAT	Antihypertensive and Lipid -lowering treatment to Prevent Heart Attack Trial (clinical trial)
ANP	Atrial natriuretic peptide
ANZ	Australia/New Zealand carvedilol trial (clinical trial)
ATLAS	Assessment of Treatment with Lisinopril and Survival (clinical trial)
AUC	Area under the curve
AV	Additional visit
β ARK	β -adrenoreceptor kinase
BD	Twice-a-day medication
BEST	Beta-Blocker Evaluation of Survival Trial (clinical trial)
B_{\max}	Maximum bound
BNP	B-type natriuretic peptide
BP	Blood pressure
bpm	Beats per minute
BRL 37344	selective β_3 -adrenoceptor agonist

CA	California
CABG	Coronary artery bypass grafting
cAMP	Cyclic adenosine monophosphate
CGP 12177	4-(3-tertiary-butylamino-2-hydroxypropoxy)-benzimidazol-2-one hydrochloride
CHF	Chronic heart failure
CI	Confidence interval
CIBIS-I	First Cardiac Insufficiency Bisoprolol Study (clinical trial)
CIBIS-II	Second Cardiac Insufficiency Bisoprolol Study (clinical trial)
COMET	Carvedilol or metoprolol European Trial (clinical trial)
CONSENSUS	Cooperative North Scandanavian Enalapril Survival Study (clinical trial)
COMT	Catechol-O-methyltransferase
COPERNICUS	Carvedilol Prospective Randomized Cumulative Survival trial (clinical trial)
DA	Dopamine
DBP	Diastolic blood pressure
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EF	Ejection Fraction
ELISA	Enzyme-Linked ImmunoSorbent assay
ENABLE	Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure (clinical trial)
eSOD	Erythrocyte superoxide dismutase

ET-1	Endothelin 1
FBC	Full blood count
FBF	Forearm blood flow as assessed by venous occlusion plethysmography
fmol	Femtomolar equivalents
GE	General Electric
GPX	Glutathione peroxidase
GSH	Reduced glutathione
Hg	Mercury
HPLC	High-performance liquid chromatography
HR	Heart rate
I	Current
IBM	International Business Machines
ICYP	Iodocyanopindolol
IDC	Idiopathic dilated cardiomyopathy
IL	Interleukin
IHD	Ischaemic heart disease
K _d	Dissociation constant
kg	Kilogramme
kHz	Kilohertz
LAS	Low amplitude signal (of signal averaged electrocardiogram)
LDL	Low density lipoprotein
LV	Left ventricle
LVEF	Left ventricular ejection fraction

μU	Microunits
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
mcg	Microgramme
MD	Doctorate of Medicine
MDA	Malondialdehyde
MDC	Metoprolol in dilated cardiomyopathy trial (clinical trial)
MERIT-HF	Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (clinical trial)
Metoprolol CR/XL	Metoprolol succinate (long-acting variant)
mg	milligramme
MI	Myocardial infarction
min	minute
mls	millilitres
mm	millimetre
MOCHA	Multicenter Oral Carvedilol Heart Failure Assessment (clinical trial)
MOXCON	Sustained Release Moxonidine for Congestive Heart Failure (clinical trial)
MOXSE	Moxonidine study (assessment of safety and efficacy) (clinical trial)
MR	Mortality rate
mV	millivolts
N	Number

NA	Noradrenaline
nmol	nanomolar equivalents
NS	Non-significant
NYHA	New York Heart Association classification system for symptoms of heart failure
OD	Once-a-day administration
OVERTURE	Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (clinical trial)
PBS	Phosphate Buffered Saline
pg	picogrammes
PRECISE	Prospective Randomized Evaluation of Carvedilol on Symptoms and Exercise (clinical trial)
QRS	Period of ventricular depolarisation on electrocardiogram
QT	Time period from start of ventricular depolarisation to end of ventricular repolarisation
RALES	Randomised Aldactone Evaluation Study (clinical trial)
RESOLVD	Randomized Evaluation of Strategies for Left Ventricular Dysfunction
RMS	Root-mean-square (of signal averaged electrocardiogram)
ROS	Reactive oxygen species
Rx	Receptor
SBP	Systolic blood pressure
saECG	Signal-averaged electrocardiogram
s.d.	standard deviation

s.e.	standard error
SNS	Sympathetic nervous system
SOD	superoxide dismutase
SOLVD	Studies of Left Ventricular Dysfunction (clinical trial)
sTNFR-I	Soluble tumour necrosis factor receptor type one
SV	Stroke volume
SVR	Systemic vascular resistance
TBARS	Thiobarbituric acid-reactive substances
TNF- α	Tumour necrosis factor
UK	United Kingdom
USA	United States of America
US Carvedilol	United States carvedilol study (amalgamated clinical study)
USCP	United States carvedilol program
V	Voltage
Val-HEFT	Valsartan Heart Failure Trial (clinical trial)
V-Heft	Veterans' Administration Vasodilator Heart Failure Trial (clinical trial)
VHeFT-II	Second Veterans' Administration co-operative Vasodilator Heart Failure Trial (clinical trial)
VMAC	Vasodilatation in the Management of Acute CHF (clinical trial)
VOP	Venous occlusion plethysmography
WBC	White blood cell
Z	Impedance
5HT1A	5-hydroxy tryptamine type 1A (serotonergic receptor type)

ABSTRACT

Routine use of beta adrenoreceptor antagonists has been the most significant advance in the management of chronic heart failure (CHF) in the past decade. This thesis compares two adrenoreceptor antagonists with proven benefit in CHF but with very different pharmacological properties.

An open label, parallel group study was designed to comprehensively compare and contrast the actions of carvedilol (17 patients) and metoprolol (12 patients) in NYHA class II and III chronic heart failure. Haemodynamic actions (during rest and adrenergic stimulation), neurohumoral effects, forearm blood flow effects, effects on cardiac electrical stability, antioxidant status, beta adrenoreceptor density, and effects on cytokines were assessed.

Mean resting heart rate reduction after 20 weeks of metoprolol (14.5 beats per minute) was greater than with carvedilol (10.1 beats per minute) but did not reach statistical significance ($p=0.12$). Metoprolol also reduced the heart rate response to dobutamine ($p=0.009$) and salbutamol ($p=0.007$) after 20 weeks of treatment, compared to baseline, an effect not seen with carvedilol.

Metoprolol reduced plasma renin concentration. Neither beta-blocker affected plasma angiotensin II concentration. Carvedilol significantly decreased plasma aldosterone concentration, and increased plasma atrial natriuretic peptide concentration, after both 10 weeks and 20 weeks. Both drugs increased plasma B-type natriuretic peptide concentrations. Neither agent significantly affected plasma endothelin concentration. Carvedilol alone significantly increased plasma norepinephrine concentration at both time points (+30.9%, $p=0.026$ and +30.7%, $p=0.046$).

Forearm blood flow (FBF) response to local high-dose dobutamine infusion was reduced by carvedilol alone ($p=0.007$). When FBF was enhanced by local high-dose salbutamol, 20 weeks treatment with metoprolol alone significantly reduced vasodilatation ($p=0.028$). Neither agent affected the reduction in FBF in response to local high dose phenylephrine nor altered the increase with local high dose acetylcholine.

QT dispersion parameters and signal averaged ECG late potentials did not alter significantly with either agent.

There was a general non-significant trend for carvedilol to increase LDL oxidation lag time, total plasma antioxidant activity and vitamin E concentration. Carvedilol also increased plasma malondialdehyde concentration ($p=0.033$) over 10 weeks, but not at

20 weeks. Metoprolol's only significant effect was reducing vitamin E concentration reaching significance after 20 weeks of treatment ($p=0.004$).

After 20 weeks of treatment, carvedilol (increasing) and metoprolol (decreasing) had divergent but non-significant effects on lymphocyte beta-receptor density. No significant effect of either agent on plasma concentrations of the cytokines TNF- α and sTNFR-I was detected in this study.

Metoprolol produced a higher degree of heart rate reduction at the doses used, while carvedilol opposed the peripheral effects of dobutamine infusion more completely. Neurohumoral differences also existed between the two agents. Carvedilol had a non-significant tendency to reduce oxidative state. These interesting differences between the agents may translate into important clinical effects.

FORMAL DECLARATION

I declare that I have written this dissertation presented to the University of Edinburgh for the degree of Doctor of Medicine; that it is based upon my own observations and that, except as acknowledged in this thesis, the data were collected and interpreted by me. This thesis has not been submitted for any other degree.

Samuel J. McClure

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Samuel J. McClure

CHAPTER 1: INTRODUCTION

1.1. HISTORY OF ADRENERGIC RESEARCH

In 1893 George Oliver, a spa physician working in Harrogate, produced extracts from adrenal glands in the belief that they might raise blood pressure and be of use in patients with hypotension. Oliver's work was probably inspired by Brown-Séquard, who had demonstrated that removal of the adrenals from experimental animals reduced their blood pressure and was invariably fatal. As a means of testing his hypothesis, Oliver took the remarkable step of giving some of the extract to his own son. Using a device of his own invention (an 'arteriometer') he showed that his son's brachial artery narrowed under the influence of the injection.(Henderson 2005)

Oliver had no means to measure blood pressure directly, so he took some of his extract to Edward Schäfer, Professor of Physiology at University College, London. Henry Dale described the meeting: 'Oliver went to tell Professor Schäfer what he thought he had observed, and found him engaged in an experiment in which the blood pressure of a dog was being recorded: found him, not unnaturally, incredulous about Oliver's story and very impatient at the interruption. But Oliver was in no hurry, and urged only that a dose of his adrenal extract, which he produced from his pocket, should be injected into a vein when Schäfer's own experiment was finished. And so, just to convince Oliver that it was all nonsense, Schäfer gave the injection, and then stood amazed to see the mercury mounting in the manometer 'til the recording float was lifted almost out of the distal limb . . .'(Dale 1948) Incidentally, the same Henry Dale is responsible for insisting on the naming of the extract "adrenaline", resulting in the British objection to the name "epinephrine" until recent European legislation was introduced.(Aronson 2000)

In 1894 Oliver and Schäfer published their findings that an extract of the adrenal gland exerted a profound effect on the heart and blood vessels in a variety of animals, producing tachycardia and vasoconstriction.(Oliver and Schäfer 1894)

By the early 1960s, agents that could antagonise the effects of “adrenaline” had been developed by Sir James Black’s group(Black and Stephenson 1962) After long term toxicity of some of the early agents was noted, propranolol was eventually developed and became the first commercially available beta-adrenergic antagonist in 1964.(Prichard 1976) Since then, many other beta-adrenergic agents have been developed, and have been used in the treatment of hypertension, control of tachycardia, and reduction of angina.

1.2. HISTORICAL ASPECTS OF THE USE OF BETA BLOCKERS IN HEART FAILURE

For many years, these beta-adrenergic antagonists (beta-blockers) were believed to be detrimental in heart failure,(Stephen 1968) as it was thought that the spontaneous enhanced sympathetic activity that occurred in heart failure was necessary to maintain cardiac output.

In January 1973 in Gothenburg, Sweden, Finn Waagstein decided to treat a 59-year-old female patient with idiopathic dilated cardiomyopathy and decompensated heart failure with intravenous practolol.(Waagstein, Hjalmarson et al. 1975) He had noted that she was very tachycardic, and was not responding to diuretic therapy. Waagstein’s rationale was that her poor cardiac function was at least partially caused by the high-energy

demand from her tachycardia.(Waagstein 1974) This daring treatment flew in the face of conventional medical thinking at the time, but was nonetheless very successful.(Waagstein, Hjalmarson et al. 1975) The patient improved dramatically, and lived to the age of 84 being treated with beta-blockers throughout the intervening years. This Gothenburg group developed the concept of heart failure treatment with beta-blockers for more than two decades before widespread acceptance occurred.

1.3. DEFINING CHRONIC HEART FAILURE

Industrialisation of societies had resulted in a world wide epidemic of chronic heart failure due to changes in life span and life style. The condition becomes increasingly prevalent with increasing age, whilst affluent lifestyles and declining activity levels have increased the prevalence of ischaemic heart disease and hypertension, the two most common precursors of the condition.

Chronic heart failure (CHF) occurs when myocardial function is impaired. A universally accepted definition of the condition has proven elusive,(Denolin, Kuhn et al. 1983) as heart failure is not a single diagnosis but is rather a recognisable collection of symptoms and signs resulting from cardiac dysfunction. Typical symptoms of CHF are breathlessness, orthopnoea, paroxysmal nocturnal dyspnoea, ankle swelling and fatigue. Typical clinical signs of CHF include tachycardia, a raised venous pressure, pulmonary crepitations and ankle oedema. Thus CHF may be regarded as a “syndrome” of recognisable symptoms and signs. This syndrome is usually progressive, resulting in serious morbidity and a poor prognosis for the sufferer.

In pathophysiological terms, heart failure is a “state in which an abnormality of cardiac function is responsible for failure of the heart to pump blood at a rate commensurate with the requirements of the metabolising tissues or to do so only from an elevated filling pressure”. Current European Society of Cardiology guidelines for the diagnosis of heart failure state that there should be appropriate symptoms present in a patient with objective evidence of appropriate underlying heart disease.(The Task Force on Heart Failure of the European Society of Cardiology 1995) “Objective evidence” generally means confirmation of cardiac dysfunction by an imaging modality such as echocardiography, fluoroscopy, or radionuclide methods.

Chronic heart failure is the final common pathway for a range of pathophysiological states but is most commonly due to ischaemic, hypertensive, or valvular heart disease. When heart failure is clinically evident (i.e. the symptoms and signs mentioned above are present) the underlying heart condition is already at an advanced stage: by this point further progression is usually inevitable. Until recent times no therapy existed which even retarded this progression, and despite advances in the understanding and management of chronic heart failure, this condition is still a major cause of morbidity and mortality in our society.

1.3.1. THE SCALE OF THE CHRONIC HEART FAILURE EPIDEMIC

In Western society, heart failure is a common and malignant condition. Epidemiological surveys estimate prevalence of CHF in North American and European populations at between 3 and 20 individuals per 1000.(Cowie, Mosterd et al. 1997) The wide variation

in prevalence estimates is probably due more to differences in methodology rather than due to large differences between populations. The prevalence in the elderly is consistently higher at 30 to 130 individuals per 1000 in those aged over 65 years.(Cowie, Mosterd et al. 1997)

Thus, ageing of our population is one of the factors causing a global growth in hospitalisation for heart failure. Better survival rates from myocardial infarction (due to the use of coronary care units, thrombolytics and revascularisation procedures) are also contributing to the overall burden of heart failure: in the past, many patients died who would now survive and will later develop heart failure. Treatments aimed at saving lives seem actually to be increasing the potential numbers of people suffering with this condition. Numbers are also increasing due to changes in classification and improved accuracy of diagnosis. This increase in heart failure admissions is occurring at a time when other cardiovascular conditions such as stroke and myocardial infarction are on the decrease. In the USA, heart failure continues to be the most common cause of hospitalisation in people over 65 years of age.(Haldeman, Croft et al. 1999)

Whether heart failure incidence is *still* increasing has been called into question by some recent studies. An assessment of long term trends in incidence from the Framingham Heart Study suggests that the incidence of heart failure has actually declined in women, though not in men.(Levy, Kenchaiah et al. 2002) This study also suggests some improvement in survival rates, though again the overall survival rate of someone diagnosed as having heart failure, even in the 1990s, was less than 50% at five years. Scottish data also supports an impact of modern treatment on the prognosis of patients suffering with heart failure.(MacIntyre, Capewell et al. 2000)

In addition to the reduced life expectancy of individuals suffering from CHF, considerable disability and morbidity are caused. This is costly because of the need for complex pharmacological therapy, community care, and especially due to frequent hospital admissions. A recent estimate suggests that 1.9% of total National Health Service expenditure in the United Kingdom is currently on CHF, of which 69% is due to hospitalisations.(Stewart, Jenkins et al. 2002)

In health care systems, hospital admissions account for a disproportionately large component of total health care expenditure. By their nature, hospital admissions due to heart failure are prolonged, and readmissions are common. Scottish figures reveal that in the early 1990s, 0.2% of the population were hospitalised for heart failure per annum accounting for more than 5% of adult general medicine and geriatric hospital admissions.(McMurray, McDonagh et al. 1993)

1.3.2. PROGNOSIS OF CHRONIC HEART FAILURE

Irrespective of its underlying cause, CHF is a serious condition indicating a poor prognosis.(Ho, Anderson et al. 1993) As might be expected, mortality in CHF is related to disease severity, and prognosis is determined largely by stage of heart failure. However, even in mild to moderate CHF, as in the “Study of Men Born in 1913”, comparative 5-year mortality was 26% in the symptomatic group compared to 8% in non-CHF men.(Eriksson, Wilhelmsen et al. 1991) In the treatment arm of the Studies of Left Ventricular Dysfunction (SOLVD) trial, 35% of the relatively young patients (mean age 61 years) with “mild to moderate” heart failure who were *treated* with

enalapril died within three and a half years of follow-up.(The SOLVD Investigators 1992)

Much of the current thrust of research in heart failure has been to improve early diagnosis of the condition. By initiating therapy before symptoms arise (during so-called *asymptomatic left ventricular dysfunction*) it is hoped that progression to the end stage of the disease is delayed or prevented. The validity of this approach depends upon the availability of therapies that are known to make a difference to prognosis, even when the condition is at an early stage; both angiotensin converting enzyme (ACE) inhibitors and more recently beta-blockers would fall into this category. ACE inhibitors have radically altered the treatment of heart failure due to left ventricular systolic dysfunction, but few patients are rendered asymptomatic by treatment with these agents or are able to lead a normal life. For this reason, more complete means of producing neurohumoral inhibition have been sought.

1.4. NEUROENDOCRINE ACTIVATION

Neuroendocrine activation is a cardinal feature of chronic heart failure and is now generally believed to promote progression of this syndrome. Much of this understanding has evolved from implementation of ACE inhibitors as therapy for CHF.(The CONSENSUS Trial Study Group 1987; Francis, Cohn et al. 1993) For the last twenty years, ACEIs have been used to modulate neuroendocrine activation in CHF by inhibiting the renin-angiotensin system. This neuroendocrine effect is crucial for the known advantages of ACEIs over simple vasodilators.(Cohn, Johnson et al. 1991)

It is proposed that additional benefit might be produced by modulation of other components of neuroendocrine activation. Sympathetic nervous system (SNS) overactivity is a particularly attractive target, with circulating plasma concentration of noradrenaline shown to be a powerful predictor of prognosis. As a means of achieving SNS inhibition, beta adrenoreceptor antagonists (beta-blockers) have now been established as an additional therapy in CHF.

A number of recent clinical trials have shown major improvements in mortality using the beta-blockers carvedilol, metoprolol and bisoprolol. These three agents (whilst all termed beta-blockers) have diverse pharmacological actions. It is not yet understood which particular properties of beta-blockers are responsible for their beneficial effects.

Carvedilol, for example, exhibits α_1 antagonism in addition to being a non-selective beta receptor antagonist, and is also a powerful antioxidant: the relevance of these known *in vitro* actions in the clinical setting of CHF is unclear. It is postulated that some of the benefit of this particular agent is more related to these properties than to pure beta-receptor antagonism. For instance, α_1 antagonism causes peripheral vasodilatation, which may offset the negative inotropism of beta-receptor antagonism; CHF has been shown to be associated with a detectable increase in the oxidant status of patients, and antioxidant activity might be a beneficial drug property. Clinically important differences between different beta blocking agents would obviously have major implications for the use of these agents in general in CHF.

By using a systematic approach to the known mechanisms of action of carvedilol, this study sets out to differentiate the pharmacological properties of this medication from

those of metoprolol. In this way we might elucidate the mechanism by which these promising agents are of assistance in the management of this important condition.

1.4.1. THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN CHF

Increased activity of the sympathetic nervous system (SNS) is an essential physiological reaction to environmental changes: even postural change would not be possible without a rapid SNS response.

Damage to the myocardium attacks the organism in a more permanent manner. Any significant myocardial damage results in a reduction in cardiac output, which is recognised by the systems that maintain cardiovascular homeostasis, and these systems attempt to correct the situation. This forlorn attempt to regain cardiovascular stability activates the SNS, the renin angiotensin system, and various other neurohumoral responses.

Unlike other situations such as hypovolaemia, where cardiac output may be *temporarily* reduced, ventricular damage is not normally reversible and neurohumoral stimulation persists. In the CHF patient, a scenario is produced in many ways similar to a normal person undertaking exercise: in the case of the heart failure patient, however, there is no period of rest for the cardiovascular system. Tachycardia, increased peripheral resistance, and salt and water retention occur and further increase cardiac workload. The counter-regulatory vasodilator, natriuretic and diuretic responses (such as increased plasma concentrations of natriuretic peptides and perhaps adrenomedullin) cannot adequately oppose the neurohumoral imbalance during permanent ventricular

dysfunction. Chronic imbalance of neurohumoral activation results in a progressive decline in circulatory performance and the clinical features of CHF. Over the last three decades it has become clear that sustained and inappropriate sympathetic nervous system activation accompanies (Chidsey, Braunwald et al. 1965; Thomas and Marks 1978; Francis, Satoh et al. 1982; Levine, Francis et al. 1982) and *contributes to* (Packer 1989; Kaye, Lefkovits et al. 1995) progression of the clinical syndrome of CHF.

1.4.2. EVIDENCE SUPPORTING THE NEUROHORMONAL HYPOTHESIS OF HEART FAILURE

The "neurohormonal hypothesis" of heart failure attributes clinical manifestations and progression of CHF to abnormal modulation of neurohumoral systems. (Francis, Goldsmith et al. 1984) Increased plasma concentrations of renin, angiotensin, endothelin and catecholamines are believed to not just accompany the condition, but are thought to perpetrate it.

Severe heart failure has been recognised as being associated with increased urinary noradrenaline excretion for many years. (Chidsey, Braunwald et al. 1965) Subsequently, elevated plasma noradrenaline concentration was reported. (Thomas and Marks 1978) It remained unclear whether these findings were simply a marker of heart failure or whether SNS activation worsened the situation.

Technical developments have subsequently enabled more "direct" methods of SNS assessment such as microneurography (Leimbach, Wallin et al. 1986) (direct intraneural recordings of e.g. peroneal sympathetic nerve traffic). Microneurography has confirmed

that SNS activity correlates with cardiac index, stroke volume index, and systemic and pulmonary vascular resistances, though is not related to ejection fraction.(Ferguson, Berg et al. 1990) Modern indirect methods of SNS assessment such as measurement of heart rate variability (Kienkle, Ferguson et al. 1992) and plasma "spill over" of noradrenaline (Esler, Jennings et al. 1988) collectively confirm that SNS activity is progressively increased, and parasympathetic activity blunted,(Eckberg, Drabinsky et al. 1971; Binkley, Nunziata et al. 1991) as left ventricular dysfunction develops into clinical CHF and this syndrome increases in severity. Despite these impressive technical achievements, an irrefutable demonstration that increased SNS output directly causes or contributes to the decline in cardiac performance and disease progression remains elusive.

Data from the Studies of Left Ventricular Dysfunction (SOLVD) registry have shown that elevated plasma concentration of noradrenaline, renin, arginine-vasopressin, and atrial natriuretic peptide are associated with a poor prognosis.(Benedict, Shelton et al. 1996) The Second Veterans Administration Co-operative Vasodilator Heart Failure Trial (VHeFT-II) has shown that elevated plasma concentrations of noradrenaline and renin are independently related to mortality and that the most beneficial response to ACE inhibition occurred in those patients with the highest pre-treatment concentrations i.e. the most neurohumoral activation.(The V-Heft VA Cooperative Studies Group 1993) This emphasises the importance of the relationship between neurohumoral activation and mortality. It also suggests that further neurohumoral blockade might produce further benefit.

The hypothesis has been bolstered by the results of several beta-blocker trials, which are discussed in further detail below. More recently, the RALES trial using spironolactone at a dose chosen to block aldosterone receptors has also proven significant mortality benefits in CHF.(Pitt, Zannad et al. 1999)

A more mature comprehension of the actions of the ACEIs is that they alter the unbalanced neurohumoral milieu from vasoconstricted, positively inotropic and fluid retaining to a more beneficial vasodilated, less inotropic and more diuretic state. In this way, cardiac workload is reduced and further decline in cardiac function is attenuated.

1.4.3. EVIDENCE THAT SYMPATHOMIMETIC AGENTS WORSEN PROGNOSIS IN CHF

It was long believed that cardiac stimulation by inotropes would support circulatory function when chronically impaired ventricular performance was present. Adrenergic activation of the failing human heart helps maintain cardiac performance over the short term by increasing contractility and heart rate. An apparently logical approach was the use of therapeutic agents to enhance endogenous adrenergic activity.

In the failing heart, β -adrenergic signal transduction (catecholamine responsiveness) is reduced secondary to desensitisation changes in β_1 and β_2 receptors, the inhibitory G protein (G_i), and by reduction in an enzyme responsible for modulating receptor activity by phosphorylation (β ARK).(Bristow 2000) There is also reduction in the expression of adenylate cyclase and specific down regulation in the numbers of β_1 receptors. In end-stage heart failure, 50%-60% of the total signal-transducing potential is lost, but

substantial signalling capacity remains.(Bristow 2000) We now speculate that these changes are in fact adaptive, as the heart tries to reduce the harmful effects of adrenergic over activity.

Trials involving several sympathomimetic positive inotropes such as dobutamine (β_1 agonist),(O'Connor, Gattis et al. 1999) dopamine agonists such as levodopa,(Shah, Amin et al. 1985) xamoterol (partial β_1 - agonist)(The Xamoterol in Severe Heart Failure Study Group 1990) and phosphodiesterase inhibitors (acting via SNS second messenger systems)(Maskin, Forman et al. 1982; Baim, McDowell et al. 1983; Shah, Amin et al. 1985; Petein, Levine et al. 1986) worsened mortality in CHF (see Table 1-1). Whilst such drugs could often improve symptoms and measures of cardiac function in the short term,(Liang, Sherman et al. 1984) they contributed to progressive decline in the long term.(Packer and Leier 1987; O'Connor, Gattis et al. 1999) In fact, no positive inotrope has so far been shown to favourably influence mortality in CHF in a significant clinical trial.

Gradually it has become clear that this approach to the treatment of CHF was fundamentally flawed. To produce long-term improvements in mortality, strategies are required which actually reduce cardiac workload, by reducing heart rate, reducing inotropic drive, reducing vasoconstriction, and permitting diuresis. Clearly, these outcomes might be expected from agents that withdraw sympathetic influences from the circulation. It is tempting to speculate that the attenuated ability of myocardium to respond to continuously high levels of endogenous or exogenous catecholamines is a natural counter-regulatory attempt to withdraw from unhelpful influences.

Table 1-1: Summary of failed sympathomimetic positive inotrope trials in chronic heart failure

DRUG	MODE OF ACTION	INOTROPISM
Xamoterol (The Xamoterol in Severe Heart Failure Study Group 1990)	β receptor agonist	++
Dobutamine (O'Connor, Gattis et al. 1999)	Dopamine, α , β agonist	+++
Ibopamine (Hampton, Van et al. 1997)	Dopamine, α , β agonist	+
Amrinone (Maskin, Forman et al. 1982)	Phosphodiesterase inhibitor	+++
Enoximone (Cowley and Skene 1994)	Phosphodiesterase inhibitor	+++
Flosequinan (Cowley, McEntegart et al. 1994)	Attenuates inositol triphosphate	+
Milrinone (Baim, McDowell et al. 1983)	Phosphodiesterase inhibitor	+++
Piroximone (Petein, Levine et al. 1986)	Phosphodiesterase inhibitor	++
Fenoximone (Shah, Amin et al. 1985)	Phosphodiesterase inhibitor	++
Levodopa (Shah, Amin et al. 1985)	Dopamine agonist	++
Vesnarinone (Cohn, Goldstein et al. 1998)	Phosphodiesterase inhibitor	++

1.5. OTHER POSSIBLE MECHANISMS OF CHF PROGRESSION

The effects of oxidative stress, (believed to increase in CHF) elevated concentrations of cytokines (mediators of inflammation known to be increased in advanced CHF) and increased apoptosis may also contribute to disease progression. As the influence of oxidative stress, cytokines and apoptosis may in part explain the adverse consequences

of excess neurohumoral activity, these areas are considered here. More detailed discussion of the influence of oxidative stress and cytokines is undertaken later in the relevant chapters.

1.5.1. THE OXIDATIVE STRESS HYPOTHESIS OF CONGESTIVE HEART FAILURE

Many disease states ranging from aging to cancer and coronary heart disease have been associated with excess free-radical activity, and antioxidants have received attention as a potential therapy for these conditions.

Oxidative stress, which may result in oxidative tissue damage, occurs when there is an imbalance between reactive oxygen species (ROS) production and antioxidant defenses such that either ROS production is increased and/or defense mechanisms are impaired.

Recently, the role of oxidative stress in CHF has been explored as a mechanism of disease progression. In the setting of CHF, excess free-radical generation may arise from many sources, including vascular nicotinamide adenine dinucleotide oxidases, xanthine oxidases, autooxidation of catecholamines, nitric oxide synthase activation, or mitochondrial leakage.(Mak and Newton 2001) Besides excess ROS generation, animal models of CHF have suggested that myocardial antioxidant defenses are also impaired.(Dhalla, Hill et al. 1996; Hill and Singal 1997) These observations have prompted the formulation of an oxidative stress hypothesis of CHF. This hypothesis states that CHF is characterized by generalized and cardiac-specific oxidative stress,

and that chronic oxidant injury contributes to impairment of myocardial function and ultimately clinical progression of CHF.

In vitro experiments have demonstrated that excess free-radical generation or impaired antioxidant function adversely affects several myocyte functions,(Rowe, Manson et al. 1983; Kim and Akera 1987; Goldhaber, Ji et al. 1989; Kaneko, Beamish et al. 1989) depresses myocardial contractility,(Blaustein, Schine et al. 1986; Schrier and Hess 1988) causes myocardial tissue injury,(Burton, McCord et al. 1984) and may also induce myocyte apoptosis.(Gottlieb, Bureson et al. 1994) *In vivo* animal models have also demonstrated the significance of oxidative injury to cardiac function; the best-studied example is that of myocardial stunning and injury due to reperfusion after a period of ischaemia.(Bolli, Zhu et al. 1987; Bolli, Zughuib et al. 1995)

Several animal models of chronic CHF have also confirmed a role for oxidative stress. Anthracycline-mediated cardiomyopathy in animals has been shown to result primarily from oxidative injury and antioxidant therapy attenuated myocardial injury in this model.(Doroshov 1983) The administration of an antioxidant acutely improved cardiac function in a dog model of tachycardia-induced cardiomyopathy providing evidence that, besides oxidant injury, oxidative stress may also reversibly depress cardiac function in this setting.(Ukai, Cheng et al. 2001)However, as will be expanded upon in section 7.2 , there is as yet no conclusive proof that oxidative stress is important in *human* CHF, nor that use of antioxidant medication can help.

1.5.2. THE IMMUNE SYSTEM AND CYTOKINES IN CHF

It is becoming increasingly apparent that inflammatory mediators play a role in the progression of CHF, to the extent that several strategies to counterbalance different aspects of the inflammatory response have recently been explored in clinical trials.(Damas, Gullestad et al. 2001) Cytokines are a family of relatively low molecular weight, pharmacologically active proteins secreted by different cell types for the purpose of altering either their own function (autocrine) or that of adjacent cells (paracrine). Cytokines implicated in the progression of CHF are tumour necrosis factor α (TNF- α), interleukin (IL) 1, and IL-6. These cytokines share some of their major characteristics, and all are proinflammatory.

TNF- α is the cytokine that has been studied in greatest detail. Amongst other actions, TNF- α has been implicated in the development of left ventricular dysfunction,(Torre-Amione, Kapadia et al. 1995) left ventricular remodelling,(Suffredini, Fromm et al. 1989) increased cardiac myocyte apoptosis,(Krown, Page et al. 1996) the development of anorexia and cachexia, reduced skeletal muscle blood flow and endothelial dysfunction,(Anker, Volterrani et al. 1998) and beta receptor uncoupling from adenylate cyclase.(Lopponow, Werdan et al. 2002)

Several hypotheses have been suggested to describe the origin of immune activation in CHF. Of note is the fact that treatment aimed at improving CHF, use of ACE inhibitors, has the effect of reducing cytokine levels.(Gullestad, Aukrust et al. 1999) Some evidence suggests that catecholamines augment this myocardial cytokine

production,(Hasko, Nemeth et al. 1998) which would obviously have great relevance with regard to this thesis.

1.5.3. APOPTOSIS AND CHF

Apoptosis and “programmed cell death” are terms used interchangeably. Apoptosis is an active, precisely regulated, energy-requiring process which appears to be orchestrated by a genetic programme.(Kerr, Wyllie et al. 1972) Apoptosis plays a crucial role in the regulation of proliferating cell populations in adult tissues and in normal tissue development.(Steller 1995) Even though cardiac myocytes are terminally differentiated, the cells still contain the genes and signal transduction apparatus for programmed cell death and thus retain the ability to die by apoptosis.(Barr and Tomei 1994) In recent years a working hypothesis has evolved that progressive left ventricular dysfunction in CHF may result in part from ongoing loss of functional cardiac units.(Sabbah 2000) Apoptosis may be the “final common pathway” of cardiac deterioration caused by various destructive influences, and thus promotes CHF progression.

This theory was based on the presence of ultrastructural degenerative changes of cardiomyocytes in failing human and animal hearts. Changes noted include myofibrillar disruption and disarray,(Sharov, Sabbah et al. 1994) disrupted internal and external membranes of mitochondria,(Sabbah, Sharov et al. 1992) hyperplasia of organelles, and abnormalities of the cardiac interstitium caused by accumulation of collagen.(Sabbah,

Sharov et al. 1995) These observations provided indirect support to the concept that ongoing myocyte degeneration and loss may occur in the failing heart.

Previous dogma stated that the heart is a terminally differentiated organ without regenerative capacity.(MacLellan and Schneider 2000) This static view of the myocardium implied that both myocyte death and myocyte replication played no meaningful role in cardiac homeostasis. In the absence of myocyte renewal, cell death by apoptosis or necrosis had to be extremely low to explain the preservation of the cardiac mass throughout the life span of the individual, as even very low rates of myocyte death would result in the complete disappearance of the myocardium. Thus, it is not surprising that the existence of myocyte apoptosis remained controversial during the past decade.(Kang and Izumo 2000) Studies in tissues obtained from explanted hearts of patients with end stage heart failure have since confirmed the presence of cardiomyocyte apoptosis.(Narula, Haider et al. 1996; Olivetti, Abbi et al. 1997)

Recent findings indicating the presence in the adult myocardium of a cell population with the behavior and potential of cardiac stem cells has challenged the status quo. One particularly intriguing report has even shown migration of cells from the recipient to the donor heart in transplant recipients.(Quaini, Urbanek et al. 2002)These findings have provided an explanation for the existence of a sub-population of immature cycling myocytes and embraced myocyte death and myocyte renewal as two aspects of cardiac homeostasis.(Nadal-Ginard, Kajstura et al. 2003) This myocyte renewal depends on the differentiation of primitive cells into immature myocytes that might divide two to four times before becoming terminally differentiated and permanently withdrawn from the cell cycle. Although there is no evidence that already mature cardiomyocytes can de-

differentiate, reenter the cell cycle, and proliferate, the heart has regenerative potential and it is not a terminally differentiated organ. In this new light, the presence, regulation, and physiological consequences of myocyte apoptosis have gained new significance.

Two recent papers highlight the effect of this type of myocyte death in cardiac performance and provide new insights on the role of myocyte death and renewal in cardiovascular physiology.(Wencker, Chandra et al. 2003; Yamamoto, Yang et al. 2003) Although it is well accepted that myocyte death is a determining factor of ventricular dysfunction and end-stage failure, whether diffuse myocyte apoptosis can, by itself, cause cardiac failure has remained controversial. These transgenic animal models of myocyte apoptosis provide conclusive proof that myocyte dropout by itself can cause cardiac failure.(Wencker, Chandra et al. 2003; Yamamoto, Yang et al. 2003) In these studies, appearance of dilated cardiomyopathy was mediated by diffuse myocyte apoptosis across the ventricular wall. Prevention of apoptosis prevented development of heart failure.

With regard to this thesis, the importance of apoptosis is in relation to the factors that can trigger this process. These factors include formation of oxygen free radicals,(Gottlieb, Burleson et al. 1994) excess levels of angiotensin-II,(Kajstura, Cigola et al. 1997) excess levels of norepinephrine (and particularly its action via β_1 adrenoreceptors)(Communal, Singh et al. 1998; Zaugg, Xu et al. 2000) and increased levels of specific cytokines such as TNF- α .(Bozkurt, Shan et al. 1996; Krown, Page et al. 1996) The underlying theme of this thesis may be to decide which of the diverse actions of the two beta-blockers under study are important to reduce the level of apoptosis in the failing heart.

1.6. STRATEGIES FOR MODULATION OF THE SNS IN CHF.

Angiotensin converting enzyme inhibitors have produced impressive benefits in heart failure prognosis, at least in part by their modulation of the renin-angiotensin-aldosterone system. Now interest has turned to methods of adjusting other neurohumoral systems and foremost potential target for pharmacological intervention is the SNS. Of note is that ACEIs themselves reduce SNS activity as measured by several means, but several other more specific methods of achieving this goal exist.

Inhibition of sympathetic traffic to the heart and peripheral vasculature may be accomplished by various means:

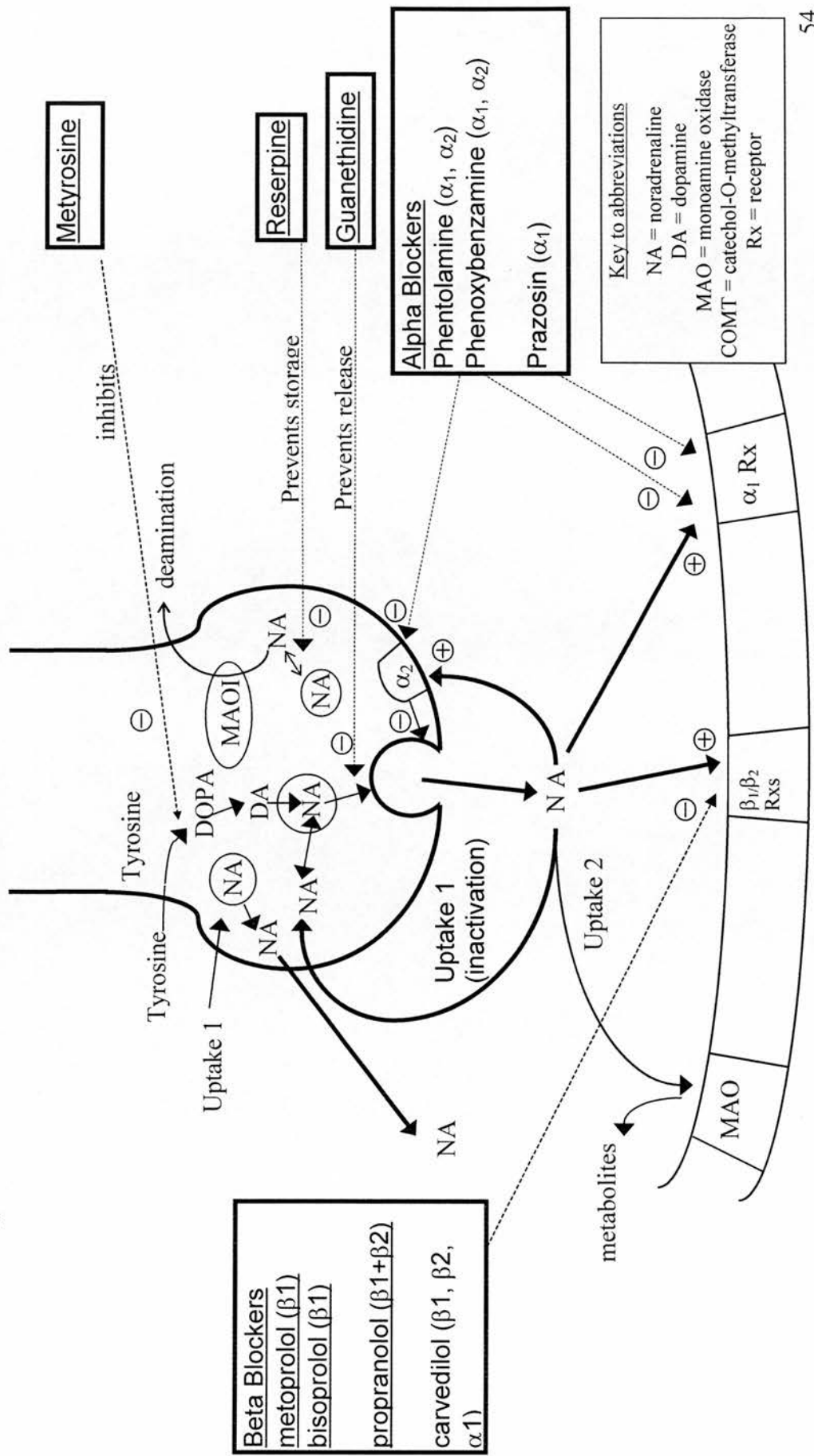
1. Blocking post-synaptic α and β receptors (as illustrated in Figure 1.1).
2. Activating central inhibitory centres using drugs such as clonidine or moxonidine.
3. Ganglion blocking drugs which interrupt transmission in the sympathetic ganglia by blockade of nicotinic cholinergic receptors e.g. hexamethonium, pentapyrrolidinium. Unfortunately, drugs in this class block almost all autonomic outflow at both sympathetic and parasympathetic ganglia, and thus produce such a wide range of side effects as to make routine use impossible. Short reports indicated a worsening in heart failure with pentapyrrolidinium. (Ronnov-Jensen 1955)

4. Depletion or inactivation of noradrenaline stores in the peripheral postganglionic sympathetic neurones using, for example, guanethidine or reserpine. These drugs also worsened heart failure in small studies.(Perera 1955; Marley and Pare 1956)

5. Inhibition of tyrosine hydroxylase to reduce production of catecholamines: one small study of the use of metyrosine for this purpose exists.(Franciosa and Schwartz 1989) Whilst a decrease in plasma noradrenaline concentration was observed, no haemodynamic effect was found and the study was taken by the authors to show no influence in CHF.

Of these options, only the first two are practical at the present time in the sense that drugs are available which are well tolerated during long term use. A diagram of the sympathetic nerve terminal, with the sites of action of several drugs, is shown below (see Figure 1.1).

Figure 1.1 Sympathetic innervation of a blood vessel, with agonists and antagonists.



1.6.1. ALPHA BLOCKERS IN HEART FAILURE

When strategies for treating heart failure were dominated by the haemodynamic hypothesis of heart failure, various different approaches to producing vasodilatation were attempted. Alpha-adrenergic receptor antagonists were employed in the hope of producing sustained vasodilatation by blocking the vasoconstriction caused by SNS overactivity.

Phentolamine and phenoxybenzamine are non-selective alpha-blockers, with action on both pre- and post-synaptic alpha receptors. Oral administration of phentolamine (Screiber, Maier et al. 1976) and phenoxybenzamine (Kovick, Tillisch et al. 1976) produced acute haemodynamic improvement in early observational reports. However, phentolamine causes reflex stimulation of the sympathetic neurones resulting in greater overall neurotransmitter release and heightened stimulation of beta-receptors, consequently accelerating heart rate. Phenoxybenzamine also produces tachycardia, by reducing presynaptic reuptake of noradrenaline (see Figure 1-1).

Short-term haemodynamic benefits were reported after administration of prazosin (Miller, Awan et al. 1977) and trimazosin, (Awan, Hermanovich et al. 1979) both selective α_1 blockers. Enthusiasm for the use of alpha-blockers faltered when it was found that tolerance to the acute haemodynamic effects developed, and there was no clinical benefit after longer-term usage. (Markham, Corbett et al. 1983) In particular, prazosin was found to produce no sustained haemodynamic or mortality benefit in CHF, (Packer, Meller et al. 1979; Packer, Medina et al. 1986) and was found to actually increase the circulating concentration of noradrenaline and to result in salt and water

retention.(Colucci, Williams et al. 1980; Stein, Henry et al. 1981) More recently, the α 1 blocker, urapidil, was tried in a small study of 36 patients with severe (NYHA III and IV) heart failure.(Dorszewski, Gohmann et al. 1997) Apart from its peripheral α 1 blockade, urapidil also has some central action due to stimulation of serotonergic 5HT1A receptors in the medulla. This agent caused an almost 5 times increased mortality rate in the urapidil arm of the study.(Dorszewski, Gohmann et al. 1997)

Doxazosin has a more prolonged duration of action than earlier members of the class, which raised the expectation that it might be more useful in CHF. However, the ALLHAT (Antihypertensive and Lipid -lowering treatment to Prevent Heart Attack Trial) study(ALLHAT Collaborative Research Group 2000) had its doxazosin-treatment arm discontinued owing to a significant *increase* in the numbers of patients developing congestive heart failure. It seems unlikely that further trials of α -blockers in chronic heart failure will be attempted.

1.6.2. BETA BLOCKERS IN HEART FAILURE

Since their development in the sixties, the conventional view of the use of beta-blockers has been that these drugs depress cardiac performance due to negative inotropy and negative chronotropy, and would thus worsen the situation in CHF. Swedish work more than twenty years ago contradicted this view,(Waagstein, Hjalmarson et al. 1975) but was not widely accepted. A number of small-scale clinical trials in the seventies and eighties were inconclusive: inotrope development was in vogue, and progress appeared to lie in this direction instead.

The enormous success of ACE inhibitors in large-scale clinical trials helped evolve the neurohormonal hypothesis of CHF and improve our understanding of this condition. This finally led to a number of clinical trials of several different beta-blocking agents in the nineties. The emphasis in these trials was not necessarily to detect an improvement in patient symptoms, at least in the short term, but was rather to detect an improvement in morbidity and mortality, which might suggest a delay in disease progression.

Most early beta-blocker trials were uncontrolled, usually assessed what might arguably be called less important parameters, or were simply under-powered to detect mortality improvements during beta-blocker therapy. The first Swedish trials were observational, (Waagstein, Hjalmarson et al. 1975) whilst during the eighties surrogate outcome parameters were employed such as exercise tolerance and change in ejection fraction. Results using different agents seemed variable, doing little to bolster the case for the use of beta-blockers in general in CHF.

Experience with ACE inhibitor trials showed that larger scale trials were required, and after the worsening mortality seen in the inotrope trials, the consensus developed that the primary end-point of trials should be influence on mortality rate. Huge numbers of patients are required for such studies, so that enough end points occur to reveal genuine differences between groups. The first of the large scale placebo-controlled beta blocker trials occurred during the early nineties, but the first which have been sufficiently powered to look at all-cause mortality have only been completed over the past few years.

1.6.3. CENTRAL INHIBITION OF SNS ACTIVITY

Centrally acting anti-adrenergic agents offer the opportunity of reducing systemic vascular resistance to offset the potential negative inotropism of withdrawing cardiac adrenergic drive. This reduced drive might be detectable by, for example, a lowered plasma noradrenaline concentration.

Centrally mediated modulation of the sympathetic nervous system has been available for many years in the form of clonidine,(Hermiller, Magorien et al. 1983; Giles, Thomas et al. 1985; Magorien, Hermiller et al. 1985; Good, Unverferth et al. 1988; Manolis, Olympios et al. 1995; Manolis, Olympios et al. 1997) α -methyldopa,(Kirlin, Das et al. 1986) guanabenz(Olivari, Levine et al. 1986) and guanfacine.(Collart, Staroukine et al. 1985) However, the concept of using these medications in the treatment of CHF has surprisingly never been subjected to significant placebo-controlled trials, despite encouraging preliminary data in these small studies.

Treatment of hypertension with centrally acting drugs originated with the use of α -methyldopa in the 1960s. This pro-drug was quickly recognised as having its predominant effect within the central nervous system. Clonidine became the main drug of this class to be used in hypertension due to its better degree of tolerability. However clonidine itself has side effects, which often make routine use unacceptable to patients: sedation (due to the central α_2 effect), dry mouth, fatigue and impotence (due to the peripheral α_2 effect) are common.

A decade ago a number of small-scale studies of clonidine in CHF were conducted, at a time when other vasodilators were becoming established. Despite some encouraging early results, there was no impetus to conduct essential larger scale trials: the "haemodynamic hypothesis" of heart failure was in vogue at the time, and seemed to explain why vasodilator medication improved the condition. The "neurohormonal hypothesis" of CHF progression was still in evolution and this, coupled with trials showing a mortality reduction with nitrates and hydralazine and thereafter with ACE inhibitors, led to a loss of interest in the results of these early clonidine trials.

The success of beta-blockers in heart failure therapy has prompted a further look at clonidine as a possible alternative means of reducing the deleterious effects of sympathetic overactivity. Moxonidine, a recently developed central SNS inhibitor, seems to avoid most of the tolerability problems associated with other centrally-acting agents, and has thus been under intense scrutiny.

An attractive feature of centrally acting anti-adrenergic drugs is that circulatory reflexes are left largely intact despite the depression of peripheral sympathetic activity. Both clonidine and moxonidine cause a fall in blood pressure, and act to reset the sensitivity of the baroreceptor reflex (which is normally reduced in CHF). This action may explain the improvement in heart rate variability (an important predictive factor for mortality in CHF) seen with both drugs.(JuulMoller, Swedberg et al. 1997) Haemodynamic studies confirm clonidine as a potential adjunctive therapy to ACE inhibitors,(Manolis, Olympios et al. 1997; Girgis, Chakko et al. 1998) and it is safe in acute dosing.(Mitrovic, Strasser et al. 1996; Dickstein, Manhenke et al. 1999)

The first large scale trial of moxonidine, the MOXCON (Sustained Release Moxonidine for Congestive Heart Failure) study was terminated early, in controversial circumstances, due to increased mortality rates in the treatment group.(Coats 1999; Cohn, Pfeffer et al. 2003) The Data and Safety Monitoring Board discontinued the study when only 2000 of the planned 4540 patients had been enrolled, due to the deaths of 53 patients in the treatment group compared to 29 in the placebo group, most deaths being sudden. There were also a greater number of hospitalisations for worsening heart failure, acute MI and other adverse events.(Cohn, Pfeffer et al. 2003) It is not yet clear why these effects have been seen, but it was noted during the study that plasma noradrenaline concentration fell to sub-physiological levels in some patients. The drug had a greater-than-expected effect on noradrenaline levels, a feature which had been noted in the preliminary MOXSE study.(Swedberg, Bergh et al. 2000) Some investigators believe that the failure of moxonidine in MOXCON has been due to the choice of an inappropriately high dose.

Given the apparent results from MOXCON the use of centrally acting agents, and particularly sustained-release moxonidine, in CHF is not currently recommended.

1.7. LARGE PLACEBO-CONTROLLED STUDIES OF BETA-BLOCKERS IN CHRONIC HEART FAILURE.

For a summary of the large placebo-controlled beta-blocker trials, see Table 1.3

1.7.1. MDC (metoprolol)

The Metoprolol in Dilated Cardiomyopathy (MDC) study (Waagstein, Bristow et al. 1993) randomised 383 patients on standard treatment to either metoprolol or placebo, and follow up was for 12-18 months. Even with this size of study, there was no significant effect on total mortality, but there was an effect which almost reached statistical significance on the combined endpoint of mortality or progression to cardiac transplantation. There was a non-significant excess of deaths seen in the metoprolol-treated group.

1.7.2. CIBIS-I (bisoprolol)

The Cardiac Insufficiency Bisoprolol Study randomised 641 patients with heart failure of various aetiologies to bisoprolol or placebo for a mean follow up period of 1.9 years: again, there was no significant difference between groups in mortality, but fewer bisoprolol-treated patients required hospitalisation, and more showed improvement in function class.(CIBIS investigators 1994) Subgroup analysis suggested that benefit from beta- blockade therapy was greater for those with non-ischaemic cardiomyopathy, which at the time of publication appeared to add support to the view that idiopathic cardiomyopathy patients had more to gain from beta-blocker therapy.

1.7.3. US carvedilol trials

This key publication persuaded large sections of the cardiology community of the value of beta blockade in heart failure.(Packer, Bristow et al. 1996) The publication was actually the amalgamated result from four smaller studies (see Table 1-2),(Bristow, Gilbert et al.; Colucci, Packer et al. 1996; Packer, Colucci et al. 1996; Cohn, Fowler et al. 1997). A total of 1094 patients were enrolled for 6.5 months, and showed a 65% relative risk reduction in mortality with carvedilol ($p=0.001$) *regardless of heart failure aetiology*. A common data and safety monitoring board monitored the collective mortality in these 4 separate trials (with separate efficacy end points) and the decision was taken to terminate the trials early due to the marked reduction in mortality seen in the treated group.

In addition to effects on mortality, carvedilol reduced the proportion of patients requiring hospitalisation for a cardiovascular cause (risk reduction 27% with 95% confidence intervals of 3-45%). The proportions admitted for any cause and for worsening chronic heart failure were also significantly reduced. Tolerability of carvedilol seems broadly similar to that of other beta-blockers in chronic heart failure, although dizziness is perhaps more common. This study was, however, an amalgam of four smaller studies, none of which had been powered to look at mortality. Follow-up was short and the number of clinical events was very small.

Table 1-2: Components of the US carvedilol programme

	Number of patients	Proportion of patients treated with carvedilol : placebo	Primary end point
Mild CHF (Colucci, Packer et al. 1996)	366	2:1	Progression of CHF
MOCHA (Bristow, Gilbert et al. 1996)	345	3:1	Exercise tolerance
PRECISE (Packer, Colucci et al. 1996)	278	1:1	Exercise tolerance
Severe CHF (Cohn, Fowler et al. 1997)	105	2:1	Quality of life

1.7.4. ANZ (carvedilol)

This study enrolled 415 patients with mild CHF and randomised to placebo or carvedilol. There was a non-significant reduction in mortality at 18 months: lack of significance has been attributed to the relatively good prognosis for patients with mild CHF.(Australia-New Zealand Heart Failure Research Collaborative Group 1995)

1.7.5. CIBIS-II (bisoprolol)

This study was the first randomised, double-blind, placebo-controlled trial of a beta blocker in heart failure with sufficient power to address all-cause mortality as a primary end-point.(CIBIS-II investigators and committees 1999) The large number of patients and the large number of deaths in the study (384 in CIBIS-II compared to 54 in the US carvedilol studies) make the data much more robust than the carvedilol studies. Due to

its robust methodology, CIBIS-II was actually the first study to provide conclusive evidence in favour of beta-blockers in heart failure. A total of 2647 ambulatory, mostly male, patients said to have NYHA grade III or IV heart failure of ischaemic or non-ischaemic aetiology and left ventricular ejection fraction $\leq 35\%$ were recruited. The study was halted early, with average follow-up of 1.3 years, due to a highly significant mortality reduction in favour of bisoprolol. A fall of 34% in all cause mortality ($p < 0.0001$) was seen, and of particular note was the 45% reduction in sudden death.

Bisoprolol also achieved a significant effect on cardiovascular morbidity, with fewer bisoprolol-treated patients (33%) requiring hospital admission as compared with placebo (39%). Hospital admission for worsening heart failure was also reduced to 12% from 18% in the placebo group. Tolerability of the medication was the same (15% discontinued) in both the placebo and the treatment groups.

1.7.6. MERIT-HF (metoprolol CR/XL)

The Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF) is the largest trial so far of beta-blockers in heart failure, and has been particularly encouraging as its results are highly consistent with those of CIBIS-II and with meta-analyses of previous beta-blocker trials. (MERIT-HF investigators and committees 1999) The 3991 patients included were in NYHA functional class II-IV, had left ventricular ejection fractions less than 40% and all were on standard heart failure therapy. Use of a long-acting form of metoprolol produced a 34% reduction in mortality after a mean follow-up time of 1 year. Again, an independent safety committee

recommended early completion of the study due to the magnitude of benefit seen in the treated group. Both sudden deaths and deaths due to heart failure were reduced. Like CIBIS-II, the large study size and large number of events (362 deaths) would suggest that the data are robust. Tolerability of the drug was very similar to that of placebo (15.3% in the placebo group discontinued because of side effects, compared to 13.9% of the metoprolol group).

1.7.7. BEST (Bucindolol)

Bucindolol is another beta-blocker with vasodilating properties, which has already been extensively evaluated in heart failure patients. Results from the BEST (Beta-Blocker Evaluation of Survival Trial) study suggest no *statistically significant* benefit of treatment on all-cause mortality.(BEST Trial Investigators 2001) This was an unexpected result, with only a non-significant trend toward benefit in patients with moderate to severe heart failure: mortality was reduced by 10% on bucindolol as compared with placebo ($P=0.109$). This magnitude of benefit is obviously considerably less than that seen in CIBIS-II and MERIT-HF. A higher proportion of patients had severe heart failure (recruitment was open to patients with NYHA class III-IV) in this study than in CIBIS II or MERIT-HF, and the ethnicity of the patients was different (whilst CIBIS II and MERIT-HF had predominantly Caucasian subjects, BEST had a 23% African-American complement). Subgroup analysis showed that in patients with NYHA III heart failure or left ventricular ejection fraction $>20\%$, improved survival occurred, but there was no benefit in those with LVEF $<20\%$ or in NYHA IV. Of particular note was a non-statistically significant *excess* mortality in the African-

American subgroup. Commentators have suggested that future studies should pay more attention to the ethnicity of the study population, as there may in fact be differences between racial groups in their response to therapy.

Bucindolol cannot therefore be recommended as a treatment option in CHF. This opens up the question as to whether the mortality benefit of beta-blockers in CHF is a “class effect”, whether there are specific properties of some beta-blockers, which are more desirable, or whether bucindolol has some peculiar adverse property. Bucindolol has intrinsic sympathomimetic activity, and comparison might be made with xamoterol, a beta-blocker with marked sympathomimetic properties which also had adverse effects on mortality when used in CHF. (The Xamoterol in Severe Heart Failure Study Group 1990)

1.7.8. COPENICUS (carvedilol)

Despite the large numbers of patients enrolled in the above studies, concern still existed about the administration of beta-blockers to those patients with more severe heart failure. Earlier large-scale studies with carvedilol, metoprolol and bisoprolol had enrolled patients with mainly NYHA grade II-III heart failure. The COPENICUS study attempted to address this issue.(Packer, Coats et al. 2001)

Patients were enrolled who had dyspnoea or fatigue at rest or on minimal exertion, and with a left ventricular ejection fraction of 25% or less. Patients were excluded if they were not clinically euvolaemic, and thus the most severely affected patients were still

excluded (those with pulmonary rales, ascites, significant peripheral oedema, those requiring intensive cardiac care, and those with a serum creatinine $>248\mu\text{mol/litre}$).

In a double blind manner, 1133 patients were assigned to placebo and 1156 to treatment with carvedilol. Over a period of 10.4 months, there were 190 deaths in the placebo group and 130 deaths in the carvedilol group, reflecting a 35% decrease in the risk of death during treatment with carvedilol. The combined endpoint of death or hospitalisation was also reduced by 24%. An additional finding was that treatment with carvedilol was very well tolerated, with fewer patients in the active treatment arm (as opposed to the placebo arm) requiring permanent treatment withdrawal because of adverse events. Despite exclusion of the most severely affected patients, the true severity of the heart failure in the patients enrolled has been verified by an annual mortality rate of 19.7% per patient year of follow-up in the placebo arm. This compares to rates of 11.0%-16.6% in the other trials above. Indeed, when subgroup analysis was performed to look at particularly high-risk patients (for example, those with recent or recurrent cardiac decompensation), even this group benefited greatly by treatment with carvedilol. Whilst not permitting the benefits of beta-blocker therapy to be extended to *all* CHF patients, the COPENICUS study certainly gave more confidence in applying treatment to much less healthy patients.

Table 1-3: summary of the large placebo-controlled beta-blocker trials

	MDC			CIBIS-I			US Carvedilol			A-NZ Carvedilol			CIBIS-II			MERIT-HF			COPERNICUS			BEST		
	pla	met		pla	bis		pla	carv		pla	carv		pla	bis		pla	met		pla	car		pla	buc	
n	189	194		321	320		398	696		208	207		1320	1327		2001	1990		1133	1156		1354	1354	
mean age (yr)	49	49		59	60		58	58		67	67		61	61		64	64		63	63		60	60	
male (%)	75	70		83	83		76	77		85	76		80	61		78	77		80	79		77	79	
NYHA I (%)	-	-		-	-		-	-		30	30		-	-		-	-		0	0		0	0	
NYHA II (%)	47	42		0	0		52	54		49	59		-	-		41	41		0	0		0	0	
NYHA III (%)	47	51		95	95		44	44		21	11		83	83		55	56		All patients			92	92	
NYHA IV (%)	4	4		5	5		3	3		-	-		17	17		4	3		NYHA III or IV			8	8	
LVEF (%)	22	22		26	25		22	23		29	28		28	28		28	28		19.8	19.9		23	23	
IHD (%)	0	0		53	56		47	47		89	88		50	50		66	65		67	67		58	59	
hypertension (%)	-	-		-	-		-	-		-	-		-	-		44	44		-	-		59	59	
AF (%)	-	-		14	12.5		-	-		-	-		20	20		17	16		-	-		12	11	
angina (%)	0	0		35	38		-	-		25	24		-	-		-	-		-	-		-	-	
ACE inhibitor (%)	82	78		91	89		95	95		85	86		96	96		90	89		97	97		91	91	
mean follow-up (mths)	16(approx)			23			6.5			19			16			12			10.4			24		
Hospitalisation (no.)	83	51		90	61		78	98		120	99		39	33		-	-		317	295		875	829	
Transplantation (no.)	12	2		6	3		-	-		-	-		-	-		-	-		-	-		41	29	
mortality (%)	11	12		21	17		7.8	3.2		12.5	9.7		17.3	11.8		11.0	7.2		16.8	11.2		16.5	15	
absolute MR red ^a	-	-		4.0			4.6			3.2			5.5			3.8			5.6			1.5		
relative MR red ^b (%)	-	-		-	-		65			-			34			34			35			9		
p value	NS	NS		NS			<0.001			NS			<0.0001			0.00009			0.0014			NS		

1.8. EFFECTS OF BETA BLOCKERS ON MORBIDITY AND QUALITY OF LIFE

Although beta-blockers prolong life, effects on quality of life and morbidity are of course very important. In the mid nineties a debate occurred after studies employing positive inotropes were found to reduce survival in chronic heart failure, but improve quality of life.(Cowley and Skene 1994; Rector, Tschumperlin et al. 1995)One side of the argument is that patients should be permitted to choose between quantity and quality of life. (Lewis, Johnson et al. 2001) Whilst patients might live longer on beta-blockers, would there be a reduction in the quality of life so as to make their use unacceptable?

Quality of life is a notoriously difficult measure to quantify due to its subjective nature. In the large beta-blocker studies, variables used to make some assessment of quality of life have included physician-assessed NYHA class and patient-assessed quality of life. Within beta-blocker trials, quality of life assessments have not given a clear answer as to whether the effect of beta-blockers is positive.(Reddy and Dunn 2000) In one meta-analysis of more than 3000 patients involved in large beta-blocker trials (which had otherwise shown a beneficial mortality effect), no clear improvement in quality of life could be discerned.(Lechat, Packer et al. 1998)Other investigators suggest that a quality of life effect is quantifiable.(Hjalmarson, Goldstein et al. 2000) In this 741 patient sub-study of the MERIT-HF trial, NYHA functional class (assessed by physicians) and McMaster Overall Treatment Evaluation score (assessed by patients) both improved in the metoprolol CR/XL group compared with

the placebo group. At present, it cannot be conclusively stated that quality of life is improved after treatment with beta-blockers.

As an indication of *morbidity*, the reduction in hospitalisation frequency is striking in both CIBIS-II and MERIT-HF. (CIBIS-II investigators and committees 1999; Hjalmarson, Goldstein et al. 2000) This variable is usually regarded as being least susceptible to observer bias, and has been accepted by some observers as being indicative of an improvement on quality of life.(Fowler, Vera-Llonch et al. 2001)

1.9. CHOICE OF BETA BLOCKER

Whether all beta-blockers have the same effect or whether there is a “best beta blocker” remains undecided. Bisoprolol, metoprolol and carvedilol are the drugs with most evidence to support their use at present. This thesis is obviously intended to explore differences between metoprolol and carvedilol.

1.10. COMET STUDY

The recent publication of the Carvedilol or Metoprolol European Trial (COMET) (Poole-Wilson, Swedberg et al. 2003) has unfortunately not helped to clarify the situation.(Dargie 2003; Massie 2003) This study was conceived as a definitive means of comparing clinical outcomes with metoprolol and carvedilol in CHF.

The multi-centre, double blind, randomised parallel group design assigned 1511 patients with CHF to treatment with carvedilol (target dose 25mg twice daily) and 1518 to metoprolol tartrate (target dose 50mg twice daily). Patients were required to

have CHF (NYHA II-IV), a previous admission for a cardiovascular reason, an ejection fraction of less than 35%, and to have been optimally treated with diuretics and angiotensin converting enzyme inhibitors unless not tolerated. The primary end points were all-cause mortality and the composite end-point of all-cause mortality or all-cause admission. The mean duration of the study was 58 months.

The composite end point of mortality or all-cause admission occurred in 1116 (74%) of 1511 on carvedilol and in 1160 (76%) of 1518 on metoprolol (a non-significant difference, $p=0.122$). An equal number of hospitalizations occurred in both groups. All-cause mortality was 34% (512 of 1511) for carvedilol and 40% (600 of 1518) for metoprolol (hazard ratio 0.83, 95% confidence interval 0.74-0.93, $p=0.0017$). This represented a 17% relative risk reduction in mortality for those treated with carvedilol as compared to metoprolol. Study drug was permanently discontinued by 32% of the subjects in both treatment groups, and the incidence of adverse effects was similar in both groups.

COMET included a large number of endpoints due to its long duration. The first patient was entered on December 1, 1996, and the last of the 3029 patients was enrolled on January 15, 1999, with a mean duration of follow-up of 57.9 months. The confidence intervals around the hazard ratio are narrow, making the effect on mortality result highly reliable. In a population of heart failure patients with a 10% expected annual mortality rate, the 17% mortality reduction observed in COMET translates into approximately 1 life saved for each 60 patients treated with carvedilol for a year compared with metoprolol in the formulation and doses studied.

The cardiology community has, however, interpreted the result from COMET as being far from conclusive. In particular, controversy exists as to whether the comparison of short-acting metoprolol with carvedilol (as opposed to the long-acting compound used in the MERIT-HF study) was appropriate. The MERIT-HF study employed an extended-release form of metoprolol succinate (metoprolol CR/XL), whilst COMET used more conventional metoprolol tartrate 50mg BD. In COMET a dose of 100mg metoprolol per day was planned (actual mean dose was 85mg). The mean daily dose of study drug in the metoprolol CR/XL group of MERIT-HF was 159 mg once daily, equivalent to 106mg of metoprolol tartrate when the different bioavailability of the slow-release form is taken into account. Thus the dose of metoprolol used in COMET was somewhat less than that shown to be effective in MERIT-HF. The mean daily dose at entry into the maintenance phase of the trial was 42 mg in the carvedilol group.

This possible lack of equivalent drug effect becomes even more important when observing the heart rate reduction in the metoprolol arm of COMET. Despite an identical mean heart rate at baseline in both treatment groups, a significant difference in mean heart rate was observed at 4 months between the 2 groups. Reductions in heart rate seen in the first few months of COMET were 13.3 bpm in the carvedilol group and 11.7 bpm in the metoprolol group, though after longer treatment the heart rate reduction in both COMET arms was virtually identical. Significant differences in heart rate reduction persisted in the trial until after 16 months of follow-up. This effect on heart rate during the first few months in COMET leaves open the possibility that some of the difference in efficacy seen was due to differing degrees of beta blockade, possibly due to inadequate doses of metoprolol.

Similarly, despite an identical mean blood pressure at baseline in the 2 groups, a significantly greater reduction in systolic blood pressure was observed at 4 months in the carvedilol group compared with the metoprolol tartrate group (3.8 versus 2.0 mm Hg, respectively). Significant differences in both systolic blood pressure and diastolic blood pressure between the 2 groups were observed throughout the trial. It is thus difficult to be certain that in COMET, metoprolol exerted a similar degree of β_1 blockade as carvedilol.

Active debate over the COMET results continues, with a number of authorities in this field having sharply contrasting views.(Massie 2003) Some observers contend that the dose of metoprolol used is the only reason for the difference seen,(Bristow, Feldman et al. 2003) and there is certainly evidence to support the theory that the only effect of a beta-blocker that matters in CHF is its β_1 receptor antagonism. Other authors maintain that the difference between the two agents must be due to the subsidiary effects of carvedilol, with either the alpha blockade or antioxidant action being the favoured possibilities.(Packer 2003) This argument is fundamental to the topic of this thesis, and will be expanded upon in the individual chapters.

The objections to the findings of the COMET study can also be levelled at the study detailed in this thesis, as compounds used and dosage regimes were based on the same original data and thus virtually identical.

1.11. BENEFICIAL MECHANISM OF ACTION OF BETA BLOCKERS

Which particular action of beta-blockers is responsible for slowing CHF progression?

Possible mechanisms include:

1. Blockade of the direct toxic effects of catecholamines (Imperato McGinley, Gautier et al. 1987; Mann and Cooper 1988) (particularly with the less selective beta-blockers).
2. Reduction of systemic vascular resistance (SVR): in the short term, most beta blockers actually increase SVR, but the SVR is decreased after long term administration (Waagstein, Caidahl et al. 1989) presumably due to improved myocardial performance or a decrease in sympathetic drive.
3. Antiarrhythmic action.(Cleland and Dargie 1988)
4. Reduced renin secretion.(Holmer, Hense et al. 1998)
5. Prolonged diastolic filling permitting increased effective myocardial blood flow.
6. Reduced myocardial ischaemia.
7. Slowing of heart rate, resulting in lower myocardial energy expenditure.

This last mechanism of action is particularly attractive, as perhaps simply "resting the weary heart" is all that is required. Increased heart rate itself is an independent risk factor for death in CHF (and indeed, in a variety of cardiovascular conditions (Palatini and Julius 1996)). The relative risk of all-cause mortality increases with increasing heart rate; this effect is restricted to cardiovascular causes of mortality and does not apply, for example, to cancer mortality. (Kristal, Silber et al. 2000) It is of note that the failed strategy of using positively inotropic medication to treat CHF produces increases in heart rate. Whether increased heart rate is simply an indicator of poorly treated CHF, or indicates increased risk, has not yet been clearly determined.

1.12. PRACTICAL CONSIDERATIONS FOR INITIATION OF TREATMENT WITH BETA BLOCKERS IN CHF

Initiation of treatment with beta-blockers in heart failure must be extremely cautious.(Eichhorn and Bristow 1997) Large-scale trials so far have used patients with stable, compensated heart failure. Beta-blockers are generally regarded as inappropriate for those with overt pulmonary or peripheral oedema. Even in the COPENICUS study, patients were required to be euvolaemic.(Packer, Coats et al. 2001)

A major issue when interpreting the results of heart failure trials is the difficulty in being certain about the severity of symptoms. Classification by NYHA class is notoriously subjective and susceptible to bias. A much better arbiter of patients' true CHF severity is to observe the mortality rate in the placebo arm of the trial. Assuming that randomisation has been appropriately conducted, this gives a much better indication of CHF severity. The great majority of patients enrolled in the large-scale clinical trials have had NYHA II or NYHA III heart failure. The US Carvedilol,(Cohn, Fowler et al. 1997) MERIT-HF(MERIT-HF investigators and committees 1999) and CIBIS-II(CIBIS-II investigators and committees 1999) studies were conducted in patients with mild to moderate heart failure, confirmed by the annual placebo group mortality rates of 7.8%, 11%, and 13.2% respectively. Among patients enrolled in these studies, 8% were said to have experienced NYHA class IV symptoms at baseline. Only with the release of the COPENICUS study results has robust data become available on patients with more severe CHF, with an annual

placebo mortality of 19.7%.(Packer, Coats et al. 2001) All patients were assessed as having NYHA class III or IV symptoms at randomisation to the trial.

It is no surprise that less well patients are more likely to develop adverse events during initiation and dose titration,(MacDonald, Keogh et al. 1999) but these less well patients may also have more to gain. Certainly NYHA II and III patients should all be treated with beta-blockers unless there are contraindications. There are no guidelines to help identify those patients most likely to have a favourable response to therapy.

What is clear is that the initial dose of beta-blocker should be low, far less than the dose used conventionally in hypertension or angina. Dose increases should be carried out under careful supervision usually at 2 weekly intervals, simply because most of the trials used a titration schedule like this. It *may* be possible to increase doses more rapidly, but no data exists on this issue. By slow up-titration, the supportive role of SNS activity for the circulation is only slowly withdrawn. Carvedilol should be started at 3.125mg BD, and bisoprolol at 1.25mg OD.

The only two beta-blockers licensed for use in CHF in the UK are carvedilol and bisoprolol, both of which are now commercially available in the doses employed in the CHF studies. Metoprolol is not licensed for CHF, and indeed the long-acting preparation used in MERIT-HF is not yet available in the UK. Whether this long-acting form is required for the beneficial effect remains open for debate,(Kukin, Mannino et al. 2000) particularly after the results of the COMET trial.

Even with careful initiation, physicians employing beta-blockers to treat heart failure must be aware that there may be short-term decompensation requiring increases in the

dose of diuretics. In the case of vasodilating beta-blockers, symptoms of hypotension may require a *decreased* diuretic dose.

1.13. EVIDENCE AGAINST THE NEUROHORMONAL HYPOTHESIS

Despite the impressive achievements in the treatment of CHF with therapies which appear to support the neurohormonal hypothesis, results of some recently completed clinical studies call aspects of this hypothesis into question.

The centrally-acting norepinephrine-lowering agent moxonidine resulted in increased mortality in the MOXCON trial.(Coats 1999; Cohn, Pfeffer et al. 2003)As sympatholysis is the only known action of moxonidine, this result challenged the whole dogma that antiadrenergic therapy is beneficial. In the BEST trial, too, sympatholysis (marked lowering of plasma norepinephrine concentration) achieved by the use of bucindolol seemed to result in increased mortality, at least in some subgroups.(Bristow, Zelis et al. 2001) The existence of polymorphisms of adrenergic receptors may explain why these subgroups fared less well during therapy. It has been suggested that when the degree of sympatholysis is overwhelming, this removes the important supportive role of the SNS in a manner that prevented adaptation. It is very likely that our current understanding of the functioning of the SNS is incomplete.

However, it has not just been with relation to the SNS that the neurohormonal hypothesis has given us apparently incomplete comprehension. The first inkling of the phenomenon of a neurohumoral “ceiling” of effect came from the ATLAS trial,(Packer, Poole Wilson et al. 1999) where no mortality benefits were seen with higher dose ACE inhibitors. Furthermore, the Val-HEFT trial(Cohn, Tognoni et al.

2001) indicated that the combination of ACE inhibition, angiotensin receptor antagonism, and beta blockade was positively harmful.

Since these studies, there have been disappointing results from further attempts at extrapolation of the neurohormonal hypothesis. The OVERTURE trial(Packer, Califf et al. 2002) tried to show that use of the neutral endopeptidase inhibitor, omapatrilat (producing ACE inhibition, but also increasing circulating plasma concentrations of natriuretic peptides which are normally degraded by neutral endopeptidase), would be more beneficial than a simple ACE inhibitor. However, there was no significant advantage with omapatrilat, and a higher rate of side effects. Trials of endothelin receptor antagonists, bosentan and enrasanten have been disappointing. In particular, bosentan failed to produce benefit in the ENABLE trial in severe CHF.(Kalra, Moon et al. 2002)

The use of the neurohormonal hypothesis has certainly been helpful in stimulating new avenues of research into the pathophysiology of CHF. However, there appears to be a limitation as to how far the limits of the hypothesis can be taken.(Massie 2002) When one attempts to inhibit too many pathways of neurohumoral activation, or when one inhibits a particular pathway too much, clinical results seem to be worse. It is certain that the complexity of neurohumoral pathways is greater than we yet understand.

1.14. CONCLUSION

Sympathetic nervous system modulation is a current frontier in heart failure treatment. There is much to commend the use of beta-blocker therapy for stable CHF patients as

a means of retarding the progression of their condition: careful introduction of beta-blockers should now be standard in management of stable CHF patients by cardiologists. The remaining question is: which beta-blocker is best?

1.15. HYPOTHESIS

Our hypothesis was that the actions of carvedilol and metoprolol in patients with New York Heart Association (NYHA) class II and III chronic heart failure could be distinguished when assessing the following parameters:

- i) systemic haemodynamics using intravenous infusions of adrenoreceptor stimulants
- ii) peripheral arterial effects using forearm venous occlusion plethysmography (response to brachial intra-arterial infusion of dobutamine, salbutamol, phenylephrine and acetylcholine)
- iii) endothelium-dependent peripheral blood flow (following temporary arterial occlusion)
- iv) neuroendocrine activity using measurement of renin, angiotensin II, aldosterone, adrenaline, noradrenaline, atrial natriuretic peptide, B-type natriuretic peptide and endothelin concentrations
- v) plasma cytokine activity using measurement of plasma concentrations of TNF- α and sTNFR-2
- vi) cardiac β_2 -adrenoreceptor density using in vitro measurement of lymphocyte beta-receptor density
- vii) antioxidant effects using LDL oxidation, plasma total antioxidant activity, plasma vitamin E concentration and measurement of plasma concentration of MDA
- viii) electrocardiographic stability using QT dispersion data and signal-averaged ECGs

CHAPTER 2: METHODS

2.1. CONTEXT OF THE STUDY

When this study was first planned, the US carvedilol program (USCP) results had just been released. (Packer, Bristow et al. 1996) Whilst controversial due to the complex and unconventional study design, the USCP results strongly suggested a beneficial action of carvedilol in CHF. An obvious question was whether carvedilol has unique properties, or whether all beta-blockers produce a reduction in mortality rate in CHF. A small-scale study such as that employed in this thesis would not be able to demonstrate this, but would enable a detailed comparison of the properties of individual beta-blockers when used in CHF.

Metoprolol was chosen for comparison with carvedilol. Carvedilol is an antagonist at various different adrenoreceptors and has *in vitro* antioxidant activity, whilst metoprolol has a much narrower range of action as a highly specific antagonist at the β_1 adrenoreceptor. Large studies have since confirmed a dramatic beneficial effect not only of carvedilol but also of modified-release metoprolol and bisoprolol in CHF.(CIBIS-II investigators and committees 1999; MERIT-HF investigators and committees 1999) The results of a large comparative study of clinical results with carvedilol and metoprolol in CHF (the COMET study) have recently been released.(Poole-Wilson, Swedberg et al. 2003)

2.2. OBJECTIVE

This open label, parallel group study was designed to comprehensively compare and distinguish the actions of carvedilol and metoprolol in patients with New York Heart

Association (NYHA) class II and III chronic heart failure. Individual objectives of the various study components are listed in the appropriate chapters.

2.3. OVERALL STUDY DESIGN

This study was conducted in accordance with recommendations guiding physicians in biomedical research involving human patients as laid down by the Declaration of Helsinki. The West Ethical Committee of Greater Glasgow Health board granted ethical approval.

Our original protocol had baseline, acute (after first dose of the study drug), sub acute (after full titration to target dose of study drug) and chronic (after maintenance at target/maximum tolerated dose of study drug) study days. It had been intended to perform forearm blood flow recordings, systemic infusions and blood sample analyses at all four of these time points. The local ethical committee advised that this would result in an unacceptable burden on the subjects involved and consequently the protocol was revised to take this into account. On the advice of the ethical committee, we restricted these more intensive study days to two in number (see section 2.4). The first was on the first day of study drug dosing, with baseline blood samples being taken, then forearm studies and systemic infusions taking place after the first (very low dose) of study drug. The second intensive study day was at the end of the maintenance phase, after twenty weeks of treatment.

Adrenergic effects of carvedilol and metoprolol on systemic haemodynamics were studied using systemic intravenous infusions of adrenoreceptor stimulants. Peripheral arterial effects were assessed by forearm venous occlusion plethysmography,

assessing forearm blood flow in response to brachial intra-arterial infusion of dobutamine, salbutamol, phenylephrine and acetylcholine. Measuring forearm blood flow response following blood flow occlusion assessed the effect of the study drugs on endothelium-dependent peripheral blood flow. Neuroendocrine activity was assessed by measurement of renin, angiotensin II, aldosterone, adrenaline, noradrenaline, atrial natriuretic peptide, B-type natriuretic peptide and endothelin concentrations. Plasma cytokine activity was assessed by measurement of plasma concentrations of TNF- α and sTNFR-2. Cardiac β 2-adrenoreceptor density was assessed indirectly by in vitro measurement of lymphocyte beta-receptor density. Antioxidant effects were measured by studying LDL oxidation, plasma total antioxidant activity, plasma vitamin E concentration and measurement of plasma concentration of MDA. Electrocardiographic stability was assessed by collection of QT dispersion data and by recording of signal-averaged ECGs. Safety was assessed using routine laboratory tests and by collection of adverse event data. Assessments during the study were made according to the schedule detailed below (see Table 2-1).

2.4. INTENSIVE STUDY DAYS

These two days were the main points at which data was collected for the study. On arrival on intensive study days, patients first underwent phlebotomy (to glean the data for neurohumoral measures, antioxidant measures, and lymphocyte beta-receptor density and cytokine measures). This was followed by ECG analysis (both 12-lead ECG for QT dispersion and signal-averaged ECG). Two hours after administration of the first dose of study drug that day, forearm venous occlusion plethysmography was performed. Three hours after the administration of the second dose of study drug,

systemic infusions of beta agonists were administered combined with haemodynamic assessment.

2.5. CHOICE OF DRUG DOSES

Drug doses used were chosen with the intention of producing an equivalent degree of beta blockade. A difficulty when embarking on a trial comparing two drugs is obviously the choice of dose. Normally the most appropriate basis for comparing *beta-blockers* is their effect to reduce heart rate. This issue is made more complex by the mechanism of action of carvedilol, as the drug has other complex actions which have the potential to affect heart rate. Most notably, the vasodilatation caused by metoprolol could have the potential to cause a reflex tachycardia. Nonetheless, in deciding dose, most large scale beta blocker trials have relied on the drug effect on resting heart rate in choosing doses for drug comparisons, even when more beta blockers with more complex actions have been employed.

In the Metoprolol Dilated Cardiomyopathy (MDC) trial, the mean metoprolol dose was 108 mg daily, with a mean heart rate reduction of 15 beats per minute, or a reduction of heart rate of 0.14 bpm/mg.(Waagstein, Bristow et al. 1993) In the US carvedilol program, the mean daily dose of carvedilol was 45 mg and the resultant heart rate reduction was 13 beats per minute, or a 0.29 bpm/mg reduction in heart rate.(Packer, Bristow et al. 1996) Thus a comparison of doses twice as high for metoprolol on a milligram basis as compared to carvedilol appeared to be reasonable. It is reassuring to note that the target doses we chose for the two agents under study were more recently employed in the large-scale COMET study, comparing clinical outcomes in CHF patients being treated with these drugs.

Starting doses of the study drugs were 3.125mg bd for carvedilol and 5mg bd for metoprolol. These doses were titrated to 25mg bd or 50mg bd, respectively, or until a maximally achieved dose has been reached by week 10, when the patients entered a maintenance period. To remain in the study, subjects had to reach at least 12.5mg bd carvedilol or 25mg bd metoprolol (and titration to 25mg bd carvedilol or 50mg bd metoprolol had to have been attempted at least once). Slow titration of beta-blockers under supervision at two weekly intervals was an essential component of the study. This method of drug introduction followed the pattern in the US carvedilol programme, and obviously greatly increased the number of patient visits.

2.6. DRUG ADMINISTRATION

Patients were initially screened for inclusion into the study using the criteria presented below (sections 2.7.1, 2.7.2). Written informed consent was then obtained from the patients who were eligible. These patients were randomised, up to 7 days later, to receive either carvedilol or metoprolol for a total of 20 weeks.

The specific study drug employed was not revealed to the patients involved (the drug bottles were labelled only as containing medication pertaining to the study, without mention of whether they contained carvedilol or metoprolol). Due to the restricted availability of usable preparations of the agents used, it was impossible to blind the investigator to the individual drug used for a particular patient. The only available preparation of metoprolol was not manufactured in a dosage small enough for appropriate comparison to carvedilol 3.125mg, and thus the scored tablets were halved. This meant that it was potentially obvious to the investigator to which drug a patient had been randomized.

2.6.1. RANDOMISATION

Randomisation of therapy was performed on the basis of a computer-generated sequence. Consecutive recruits to the study were assigned to the next treatment (either carvedilol or metoprolol) as had been generated from the random sequence (see Table 2-2). Characteristics of all patients randomised and who completed the study treatment as planned are listed below (see Table 2-3, Table 2-4, Table 2-5).

2.6.2. UPTITRATION

Up-titration visits involved patients attending for a clinic assessment when the opportunity to report any adverse events was provided. Compliance was checked at each visit by counting the tablets remaining in the bottles used for drug dispensing. Compliance is recorded below (see Table 2-6). Heart rate, blood pressure and a clinical examination for signs of heart failure were recorded at each up-titration visit. The next-higher dose of the study medication was then administered and the patients were observed in a clinic setting for two hours. Pulse and blood pressure were recorded at 30-minute intervals during this observation period. In the event, all patients tolerated the increased dose of beta-blocker administered at these visits without difficulty.

Having achieved the target at ten weeks, patients were then maintained on their maximally achieved dose for 10 weeks. Chronic responses were assessed at maintenance week 10 i.e. after twenty weeks of treatment.

Table 2-1 Overall study schedule

ASSESSMENT	INITIATION AND TITRATION PERIOD										MAINTENANCE	Follow up
	Screening day (-7 to -1)	Day 0	WEEK 2	WEEK 4	WEEK 6	WEEK 8	WEEK 10				WEEK 20	
Consent	X											
Medical history	X											
Concomitant medication check	X	X	X	X	X	X	X				X	X
Cardiopulmonary examination	X	X	X	X	X	X	X				X	
Vital signs (HR,BP)	X	X	X	X	X	X	X				X	X
Weight	X		X	X	X	X	X				X	
ECG+saECG	X										X	
NYHA class assessment	X		X	X	X	X	X				X	X
Antioxidant tests	X										X	
Neuroendocrine tests	X										X	
Lymphocyte beta receptor density	X										X	
TNF- α and TNF- α soluble receptors	X										X	
Adrenergic effects (intra-arterial infusions + intravenous infusions)		X									X	
Bioimpedance (Cardiac output) monitoring		X									X	X
FBF following occlusion	X										X	
Routine blood tests: FBC, renal function	X										X	
Adverse events assessment		X	X	X	X	X	X				X	X
Compliance assessment		X	X	X	X	X	X				X	

Key: HR=heart rate; BP=blood pressure; ECG=electrocardiogram; saECG=signal-averaged electrocardiogram; NYHA=New York Heart Association heart failure functional class; TNF=tumour necrosis factor; FBF=forearm blood flow, FBC=full blood count

Table 2-2 Randomisation sequence

Patient number	Medication	Patient number	Medication
01	Metoprolol	18	Metoprolol
02	Metoprolol	19	Metoprolol
03	Carvedilol	20	Carvedilol
04	Carvedilol	21	Carvedilol
05	Metoprolol	22	Metoprolol
06	Carvedilol	23	Carvedilol
07	Carvedilol	24	Metoprolol
08	Metoprolol	25	Carvedilol
09	Metoprolol	26	Metoprolol
10	Carvedilol	27	Metoprolol
11	Metoprolol	28	Carvedilol
12	Carvedilol	29	Metoprolol
13	Metoprolol	30	Metoprolol
14	Metoprolol	31	Carvedilol
15	Carvedilol	32	Carvedilol
16	Carvedilol	33	Carvedilol
17	Carvedilol	34	Carvedilol

2.7. STUDY POPULATION

Patients were recruited from general cardiology outpatient clinics at the Western Infirmary, Glasgow and at the Southern General Hospital, Glasgow. Informed consent was obtained from all patients prior to study entry.

2.7.1. INCLUSION CRITERIA

Patients were recruited into the study if they were of at least 18 years of age and male or female without child-bearing potential (post-menopausal) with stable chronic symptomatic heart failure (NYHA class II-III). The criteria listed below had to be met:

- At least a two month history of heart failure

- An ejection fraction of <45% documented by echocardiography within the six months prior to screening. If more than one assessment of ejection fraction had been taken, the highest recorded value was taken to represent the true ejection fraction.
- Unmodified cardiovascular medication for 4 weeks prior to baseline/screen (except for beta blockers)
- Four weeks prior to screen free of hospitalisations
- Written informed consent to participate in the study

2.7.2. EXCLUSION CRITERIA

The following were regarded as exclusions from the study:

- Patients requiring continuous or intermittent outpatient inotropic therapy
- Uncorrected haemodynamically significant primary obstructive or severe regurgitant valvular disease, non-dilated (restrictive) or hypertrophic cardiomyopathies.
- Symptomatic ventricular arrhythmias not controlled by an allowed anti-arrhythmic drug or an implantable defibrillator.
- A myocardial infarction in the past two months prior to study entry
- Unstable angina
- Active myocarditis
- Atrial fibrillation, sick sinus syndrome or second or third degree heart block with or without the presence of a pacemaker.

- Supine systolic blood pressure < 85 mmHg; uncontrolled hypertension (i.e. SBP greater than 180 mmHg or DBP greater than 110 mmHg)
- Heart rate less than 62 beats per minute (sitting)
- Current clinical evidence of obstructive pulmonary disease (e.g. asthma or bronchitis) requiring inhaled or oral bronchodilator or steroid therapy
- Evidence of significant disease that could impair absorption, metabolism, or excretion of orally administered medication, i.e. renal disease (serum creatinine >221 µmol/l or 2.5 mg/dl or creatinine clearance <25 ml/min); clinically significant hepatic disease (by laboratory i.e., ALT or AST levels greater than three times upper limit of normal range or by clinical assessment); or chronic biliary disorders.
- Platelet count less than 100,000/mm³ or WBC count less 3,000/mm³
- Brittle insulin-dependent or poorly controlled non-insulin-dependent diabetes mellitus (repeated episodes of keto-acidosis and/or hyperglycaemic coma and/or hypoglycaemic shock)
- Any condition of sufficient severity to impair co-operation in the study
- History of chronic alcohol excess
- History of drug sensitivity or allergic reaction to alpha- or beta-blockers
- Use of an investigational drug other than carvedilol within 30 days of entry into the study or within 5 half-lives of the investigational drug (the longer period will apply).
- Use of any of the following medications during the study period:
 - Monoamine oxidase inhibitors (MAOIs)
 - Non-dihydropyridine calcium channel blockers

- Any Class I or III antiarrhythmic except amiodarone
- Alpha-blockers
- Labetalol
- Beta blockers*
- Warfarin

*Patients taking beta-blockers were permitted to continue this treatment until 1 week prior to the screening assessments. This issue is discussed in detail in chapter 9 (section 9.4).

2.8. SUBJECTS STUDIED

This study was started with the intention of having sufficient patients with stable chronic heart failure (NYHA class II-III) to ensure that 15 patients in each treatment group completed the study. Initially eighteen patients were randomised to treatment with carvedilol, and fifteen were randomised to treatment with metoprolol. There were 29 male subjects and 4 female subjects. The mean age of study participants was 66.7 years. All subjects were Caucasian. Twenty-nine of the subjects were assessed as having NYHA II heart failure at study commencement; the other 4 subjects were assessed as having NYHA class III heart failure.

Seventeen patients treated with carvedilol and 12 taking metoprolol completed the study as planned. One patient treated with carvedilol suffered a fatal cerebrovascular accident during the course of the study. Two patients randomised to metoprolol had vasovagal reactions during their baseline venesection and did not continue further in the study. One further patient randomised to metoprolol withdrew after one week's

treatment for personal reasons. Further data was not obtainable because of logistical problems related to sample processing: vitamin E and LDL oxidation results were eventually obtained in 10 patients from each treatment group.

2.8.1. DEMOGRAPHICS

Table 2-2: Baseline demography: all patients randomised by treatment

Parameter		Carvedilol	Metoprolol
AGE			
Less than 65	N (%)	6 (33)	6 (40)
65 or more	N (%)	12 (67)	9 (60)
AGE			
Mean		66.8	66.6
s.d.		7.6	10.7
Range		45.9-75.6	43.9-78.5
GENDER			
Male	N (%)	16 (89)	13 (87)
Female	N (%)	2 (11)	2 (13)
RACE			
Caucasian	N (%)	18 (100)	15 (100)
WEIGHT (Kg)			
Mean		73.9	75.6
s.d.		7.5	18.5
Range		58.0-87.6	53.3-115.8
HEIGHT (cm)			
Mean		170.4	164.7
s.d.		4.8	6.3
Range		164.0-180.0	149.0-175.0
Maximum		NYHA Class	
II	N (%)	15 (83)	14 (93)
III	N (%)	3 (17)	1(7)
LEFT VENTRICULAR EJECTION FRACTION (%)			
Mean		27.8	30
s.d.		7.3	7.9

Table 2-3: Relevant medical history of randomised patients

		Carvedilol	Metoprolol
Ischaemic heart disease	N (%)	17 (94)	15 (100)
Previous MI	N (%)	13 (72)	12 (80)
Previous CABG	N (%)	4 (22)	3 (20)
Cardiomyopathy	N (%)	1 (6)	0 (0)
Hyperlipidaemia	N (%)	9 (50)	4 (27)
Peripheral Arterial Disease	N (%)	3 (17)	2 (13)
Diabetes (type II)	N (%)	1 (6)	0 (0)

Table 2-4 : Prior Medication

Medication	Carvedilol		Metoprolol	
	N	(%)	N	(%)
Total Patients Randomised	18	(100)	15	(100)
Total Patients Receiving Prior Medication	18	(100)	15	(100)
ACE inhibitors	18	(100)	14	(93)
Antianginal agents	7	(39)	5	(33)
Isosorbide dinitrate	0	(0)	1	(7)
isosorbide mononitrate	4	(22)	3	(20)
nitroglycerin	4	(22)	3	(20)
Beta-blockers	2	(11)	6	(40)
atenolol	1	(6)	2	(13)
bisoprolol	1	(6)	2	(13)
metoprolol	0	(0)	2	(13)
Calcium channel blockers	4	(22)	3	(20)
amlodipine	4	(22)	2	(13)
nifedipine	0	(0)	1	(7)
Diuretics	13	(73)	10	(67)
amiloride/frusemide	1	(6)	2	(13)
frusemide	12	(67)	9	(60)
Gliclazide	1	(6)	0	(0)
Statins	10	(56)	4	(27)
pravastatin	1	(6)	1	(7)
simvastatin	9	(50)	3	(20)
Aspirin	17	(94)	15	(100)

Table 2-5 : Study medication compliance

Compliance		Carvedilol	Metoprolol
Could not be evaluated	N	0	2
<80%	N	0	1
80% to 120%	N	15	11
>120%	N	3	1

Table 2-6 : Number of patients withdrawn from study by treatment and phase

Reason for withdrawal		Carvedilol	Metoprolol
Patients withdrawn from study		1	3
Up-titration withdrawals: Adverse experience	N	0	1
Other	N	0	2
Maintenance withdrawals: Adverse experience	N	1	0

2.9. METHODS: HAEMODYNAMIC MEASURES

2.9.1. DRUG INFUSIONS AND THEIR TIMING

The timing of drug infusions with respect to administration of beta-blockers deserves specific attention. The infusions were administered in the afternoon of intensive study days i.e. Day 0 and after 20 weeks of treatment, and on each occasion the infusions were carried out 3 hours after the second dose of study drug on that particular day. This issue of the timing of the systemic infusions will be examined further in section 4.4.

Infusions of dobutamine and salbutamol were used to achieve systemic β_1 receptor and β_2 receptor activation respectively. These are readily available beta-receptor agonists that are used clinically, and each has reasonable specificity for activation of the individual receptor subtypes. Doses of dobutamine and salbutamol were prepared (taking into account the weight of the subject) to enable infusions at the rates of 0.625, 2.5 and 10 $\mu\text{g/kg/min}$ (dobutamine) and 0.0125, 0.05 and 0.20 $\mu\text{g/kg/min}$ (salbutamol). The doses of dobutamine chosen were based on those used in certain clinical situations, in particular a dose of 20 $\mu\text{g/kg/min}$ is used during dobutamine stress echocardiography to produce an increase in heart rate and cardiac work so as to provoke cardiac ischaemia. The doses of salbutamol used were based on previous investigators results. (Gibson and Coltart 1970) The relevant drug was then infused at the three ascending doses at 5-minute intervals. Washout between the drugs, using physiological saline at 5ml / minute, was for 10 minutes.

2.9.2. PATIENT PREPARATION

The subjects were allowed to lie supine and 1% lignocaine was used to anaesthetise the skin over the antecubital fossa of the non-dominant arm. A sixteen gauge intravenous cannula with injection ports was placed into the antecubital vein. A second cannula was already present in the dominant arm from the blood sampling performed earlier in the day. Physiological saline was infused at 5ml/minute for 30 minutes into the non-dominant arm to permit equilibration.

2.9.3. MEASUREMENTS

We collected heart rate, systolic and diastolic blood pressure, left ventricular stroke volume and cardiac output data during this study. Heart rate and blood pressure readings were assessed using a “Dinamap” automated blood pressure recorder (GE Healthcare, Chalfont St. Giles, United Kingdom). Cardiac output and stroke volumes were assessed using an “NCCOM 3” bioimpedance cardiography monitor (Bomed Medical Manufacturing Ltd, Cheshire, UK), as described below (section 2.9.4). These parameters were assessed at baseline and during systemic infusion of the incremental doses of salbutamol and dobutamine (as described in section 2.9.1).

2.9.4. TRANSTHORACIC IMPEDANCE CARDIOGRAPHY

Of major concern with using beta-blockers, particularly in CHF patients, is the possible deleterious effect of these medications on cardiac output. In addition, any beneficial effect of long term beta blockade would be of great interest. The technique of impedance cardiography is a convenient and non-invasive means of assessing

cardiac output, and was therefore employed in this study. The principles behind this technique are discussed in section 4.2. Briefly, the technique requires attachment of eight standard ECG gel electrodes to the subject's thorax, four at neck level and four at diaphragm level. The bioimpedance cardiography monitor computes an estimate of cardiac output based on fluctuation in transthoracic impedance, heart rate, and patient data (gender, height and weight).

2.9.5. SPECIFIC PRECAUTIONS

As there was a small risk of precipitating cardiac arrhythmias when administering adrenergic stress, a standard cardiac ECG monitor was also attached to the patient as a safety precaution, with the intention of terminating the study if any arrhythmia occurred. Monitoring was continued for 30 minutes after completion of the study protocol for safety reasons. In the event, no such arrhythmia occurred during the study.

2.10. METHODS: PERIPHERAL ARTERIAL MEASURES

Beta-blockers are known to influence peripheral arterial tone, and peripheral arterial tone is altered in CHF. It is thus of interest to know the differential effects of metoprolol and carvedilol on this parameter.

One method of comprehensively assessing the receptor-specific actions of antagonist drugs (in this case metoprolol and carvedilol) is to attempt to activate these receptors (in this case α_1 , β_1 and β_2 receptors) in the presence of the antagonists using specific

agonists. This is a widely used approach in pharmacological studies. Thus one can discern the individual degrees of antagonism of metoprolol and carvedilol at these receptors.

2.10.1. VENOUS OCCLUSION PLETHYSMOGRAPHY

Measurement of the action of these agents on forearm blood flow was by forearm venous occlusion plethysmography performed two hours after administration of the beta-blockers on each of the two intensive study days. Precedent exists for the assessment of the effects of carvedilol at this time interval.(Morgan, Snowden et al. 1987; Hryniewicz, Androne et al. 2003) The principles of venous occlusion plethysmography are described in detail in section 5.3. Briefly, this technique allows *in vivo* assessment of the action of vasoactive drugs by measuring small changes in forearm size caused by changes in arterial in-flow to the forearm.

We infused the agonist drugs into the non-dominant brachial arteries of our patients who had either just started treatment with the beta-blockers or after 20 weeks of treatment. As explained in section 2.3, a more detailed investigation had been originally intended but was abandoned due to ethical considerations.

The agents used were dobutamine (relatively specific β_1 receptor agonist), salbutamol (β_2 receptor agonist) and phenylephrine (α_1 agonist). We also infused acetylcholine, which produces endothelium-dependent vasodilatation, to decide if either metoprolol

or carvedilol produced some of their action via an endothelium-dependent mechanism.

Finally, reactive hyperaemia was used as another assessment of endothelial function: application of ischaemia to the forearm results in a hyperaemic response, which can also be measured by forearm venous occlusion plethysmography.

2.10.1.1 EQUIPMENT SET-UP

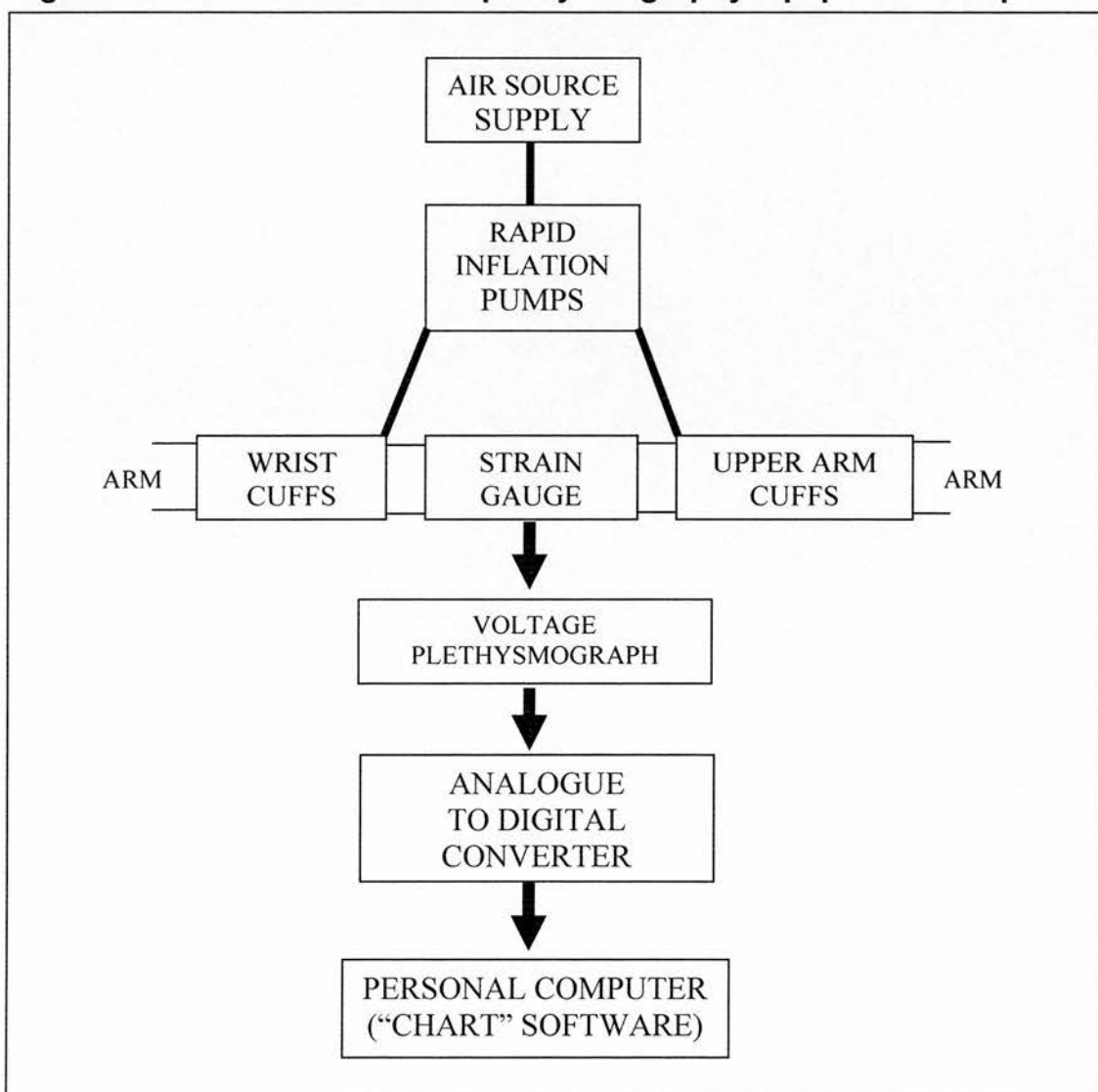
The hands were excluded from the circulation during measurement period by application of tight wrist cuffs (Hokanson SC5, PMS instruments, Maidenhead, Berkshire) that were inflated to suprasystolic pressure; hand blood flow is predominantly through the skin and is therefore not representative of the rest of the body.

Arrest of forearm venous return was achieved by inflating an upper arm cuff (Hokanson SC10, PMS instruments) to 40 mm Hg for 10 seconds, which does not alter arterial inflow or pressure. The pressure cuffs were connected to a pair of rapid cuff inflators (Hokanson E20, PMS instruments) connected to an air source (Hokanson AG101, PMS instruments). An air inflation system (rather than a fluid inflation system) was necessary to enable rapid inflation and deflation (Hokanson AG101, PMS instruments) (see Figure 2-1).

Rate of swelling of the forearm (change in forearm circumference) in millimetres per minute was measured. This was achieved using a strain gauge (Indium/gallium in silastic, Hokanson forearm set, PMS instruments) placed around the forearm at about

a third of the way from the elbow to the wrist (at the widest point of the forearm). A voltage plethysmograph (Hokanson plethysmograph, PMS instruments) accepted the signal from the strain gauge, which was transferred via an analogue-to-digital converter (MacLab 4e, AD Instruments, Hampstead, London) to a personal computer (PowerMac, Apple Computer Incorporated, USA). The data was plotted using the Chart software programme (Chart version 3.2.8, AD Instruments) and basic analysis carried out using Excel software (Excel version 7.0, Microsoft Corporation, USA). Further statistical analysis was performed using the Minitab statistical software package (Minitab Inc.) on an IBM-compatible personal computer.

Figure 2-1 : Venous occlusion plethysmography equipment set-up



2.10.1.2 PATIENT PREPARATION

Of major importance in performing the study was to ensure that arterial blood pressure (and hence forearm perfusion pressure) did not vary. The subject attended having abstained from alcohol, caffeine and tobacco for 24 hours, from food for 6 hours, and having emptied their bladder. Subjects thus abstained from any potentially vasoactive stimulants, and digestion was not active (so as not to divert blood flow from the limbs). Subjects were made comfortable and relaxed in a quiet temperature controlled room at 24°C. As far as possible, sudden noise was avoided as this can also alter blood flow.

The patient under study lay on a comfortable bed for at least 20 minutes before any drug infusion while haemodynamics adjusted to the supine position. The subject's forearms were supported on foam pads above the level of the right atrium, and the cuffs and strain gauges were applied. After local anaesthesia with 1% lignocaine (Pheonix Pharma, Gloucester), a 27 standard wire gauge steel needle (Terumo Medical Corporation, Tokyo, Japan) was inserted into the non-dominant brachial artery. The non-dominant arm was used so as to minimise the consequences in the event of complications. A constant rate infusion pump was used (IVAC P1000, Alaris Medical Systems, San Diego, California, USA) via a 16-gauge epidural catheter (Portex Limited, Hythe, Kent). Physiological saline (0.9%, Baxter Healthcare Corporation, USA) was infused for 20 minutes before drug infusion, enabling equilibration of forearm blood flows after needle insertion.

2.10.1.3 MEASUREMENTS

To measure blood flow, the wrist cuffs were inflated to suprasystolic pressure (220 mmHg) for 2½ minutes whilst the upper arm cuffs were repeatedly inflated to venous occlusion pressure (40 mmHg) for about 10 seconds and deflated for about 5 seconds. Of the resultant 9 or so separate plethysmographic recordings from each arm the first minute or so of each set were disregarded as forearm blood flow adjusted to wrist arterial occlusion. Thus 5 recordings were available for analysis. A schematic representation of the resultant plethysmographic recordings that are captured by the Chart software is shown in Figure 5-2.

After measuring the gradient of slope within the Chart programme, the data was converted to a spreadsheet of data within Microsoft Excel. Gradients due to forearm circumference changes in the infused arm were compared to those in the non-infused arm in the form of ratios, and then analysed using the Minitab statistical software package (Minitab inc.) on an IBM compatible personal computer.

2.10.2. ISCHAEMIC VASODILATATION

Ischaemic vasodilatation is an additional assessment of endothelial-dependent vasodilatation, and is performed with a near-identical set up in terms of equipment and subject preparation. The main difference is that no drug is infused during this assessment.

We performed ischaemic vasodilatation after the drug infusion protocol had been completed, and after the brachial cannula had been removed. The upper arm cuff was inflated for 10 minutes to induce forearm ischaemia. Previous investigators have reported that this duration is well tolerated and yet results in a maximal ischaemic stimulus to hyperaemia.

Due to the mild discomfort of this procedure, it was felt appropriate to perform only a single recording on each patient (on day one of the study, repeated after 20 weeks). This meant that accurate recording was essential. On a few occasions it proved impossible to achieve good quality recordings, which were free of artefact, and thus the numbers of subjects in this part of the study are small. Eight patients completed in the carvedilol group and five in the metoprolol group.

As soon as the upper arm cuff was released, forearm blood flow was recorded for the next 3 minutes or so in a manner similar to that described in section 2.10.1.3.

However in this component of the protocol repeated measurements were made throughout the 3 minutes, rather than stopping after 5 tracings. To measure blood flow, the wrist cuffs (which had been deflated during the induction of ischaemia) were inflated to suprasystolic pressure (220 mmHg) whilst the upper arm cuffs were repeatedly inflated to venous occlusion pressure (40 mmHg) for about 10 seconds and deflated for about 5 seconds. The ischaemia is an intense stimulus for blood flow, which becomes maximal immediately on release of the upper arm cuff and then quickly returns to normal levels.

After capturing the data in the Chart software, a ratio of forearm circumference in the infused arm compared to that in the non-infused arm could be calculated. Plotting of these ratios at the various time points after release of occlusive pressure (out to 3

minutes) allows construction of a curve. The larger the area under the curve in any resultant results, the slower the arm has been to recover from the hyperaemia. An increase in AUC after administration of an agent would suggest that agent had impaired the endothelial function.

2.10.3. PROTOCOL LIMITATION DURING PERIPHERAL ARTERIAL MEASUREMENTS

This component of the overall study was severely hampered by factors out with the investigator's control. Due to a major adverse event involving forearm intra-brachial infusions occurring in an entirely separate study, a temporary local ban was implemented by the West Ethical Committee of Greater Glasgow Health board on the use of the technique for further investigations.

The timing of this interruption was unfortunate, as the protocol required completion of the second forearm study day within a limited period of time. This greatly reduced the number of patients who completed the entire study protocol. This factor, in addition to a few patients being unable to comply with the full protocol, meant that only 10 patients completed in the carvedilol-treated arm and only 6 patients completed in the metoprolol-treated arm. Only those patients who had complete sets of data from both study days had their data analysed. For the reactive hyperaemia protocol, only 8 patients in the carvedilol arm completed both studies and only 5 in the metoprolol arm.

2.11. METHODS REQUIRING BLOOD ANALYSIS

Analysis of blood samples was required for neurohumoral measures, antioxidant measures, cytokine measures and for measurement of lymphocyte beta receptor density.

2.11.1. SAMPLING

Blood sampling was performed at three points during the study:

- Just prior to initiation of first dose of beta-blocker therapy
- After 10 weeks (at the end of the titration period)
- After 20 weeks (at the end of the maintenance period)

Subjects attended the study day having abstained from alcohol, caffeine and tobacco for 24 hours, from food for 6 hours, and having emptied their bladder. Under local anaesthesia (to minimise neurohumoral activation) a 16-gauge cannula was sited in the antecubital vein of the dominant arm to enable blood sampling. This cannula was also employed later the same day as part of the haemodynamic measures component of the study (see section 2.9.2). The subjects were then allowed to lie supine on a couch for 20 minutes for equilibration to occur: this is important to prevent postural variation in neurohumoral activation, which could theoretically invalidate the results of samples taken. Blood samples were then taken into chilled tubes containing the appropriate preservatives for neurohumoral tests and antioxidant tests.

2.11.2. NEUROHUMORAL ASSAYS

Dianne Hillyard performed these assays in the laboratory of Dr Ian Morton in the department of Medicine and Therapeutics at the Western Infirmary, Glasgow.

2.11.2.1 NATRIURETIC PEPTIDES

Brain natriuretic peptide was assayed without prior extraction of plasma by means of a direct, specific monoclonal antibody radioimmunoassay kit supplied by Shionogi & Co, Ltd (Settsu-shi, Osaka, Japan), as previously described.(Murdoch, Byrne et al. 1997) Plasma N-terminal atrial natriuretic peptide (ANP 1-30) was assayed by radioimmunoassay after extraction from acidified plasma with C18 reverse-phase columns (Sep-Pak, Waters Associates Ltd, Watford, UK) with the use of a modification of a previously described method. (Richards, Tonolo et al. 1987; McDonagh, Robb et al. 1998) A Peninsula Laboratories antibody, RAS 9129 (Belmont, California, USA), was used for identification of N-terminal atrial natriuretic peptide. The between-assay coefficients of variation were 15% for the N-terminal atrial natriuretic peptide assay and <10% for the brain natriuretic peptide assay, respectively.

2.11.2.2 PLASMA RENIN ACTIVITY

Plasma renin activity was measured by an in-house antibody trapping technique in the presence of added excess renin substrate.(Millar, Leckie et al. 1980) The coefficient of variation was 3.4%.

2.11.2.3 ANGIOTENSIN II

An in-house radioimmunoassay was used for plasma angiotensin II as previously described.(Morton and Webb 1985) Angiotensin II was preextracted from plasma before assay. The coefficient of variation was 10%.

2.11.2.4 ALDOSTERONE

Plasma aldosterone was measured with a solid-phase (coated tube) radioimmunoassay kit supplied by Diagnostic Products (UK) Ltd. The coefficient of variation was <8.3%.

2.11.2.5 NOREPINEPHRINE

Plasma norepinephrine (nmol/l) was extracted from plasma and assayed by high performance liquid chromatography and electrochemical detection as previously described.(Goldstein, Feuerstein et al. 1981; McAlpine, Morton et al. 1988) The within assay and between assay coefficients of variation are both < 10%.

2.11.2.6 EPINEPHRINE

Plasma epinephrine was extracted from plasma and assayed by high performance liquid chromatography and electrochemical detection as previously described.(Goldstein, Feuerstein et al. 1981; McAlpine, Morton et al. 1988) The within assay and between assay coefficients of variation are both < 10%.

2.11.2.7 ENDOTHELIN

Plasma endothelin concentration was measured by the method of Davenport et al. (Davenport, Ashby et al. 1990)as previously described.(McMurray, Ray et al. 1992) The lower limit of detection of the assay is 0.5pmol/l.

2.11.3. ANTIOXIDANT ASSAYS

Three assays were used to assess oxidant status by assessing the condition of antioxidant defences, and one assay (malondialdehyde) was used to indirectly assess oxidant status (see explanation in section 7.2.3).

2.11.3.1 LAG TIME TO LDL OXIDATION

The lag-time to induction of low-density lipoprotein oxidation was employed, as previously reported, (Godfrey, Stewart et al. 1994) to assess the inherent anti-oxidant resistance of plasma to an external oxidant (copper chloride).

2.11.3.2 PLASMA VITAMIN E CONCENTRATION

Plasma concentration of the endogenous anti-oxidant vitamin E was also measured as this varies according to the level of oxidative stress (increased oxidative stress reduces vitamin E concentration). Vitamin E (α -tocopherol) was measured by high performance liquid chromatography with ultraviolet detection at 295 nm using a commercial kit (Bio-Rad Laboratories Ltd., Hemel Hempstead, Hertfordshire UK). Intra-assay and inter-assay variability was $\leq 8\%$.

Both of the above assays were analysed in the department of biochemistry at the Western Infirmary, Glasgow, under the auspices of Dr Marek Dominiczak. Dianne Hillyard performed the next two assays in the laboratory of Dr Ian Morton in the department of Medicine and Therapeutics at the Western Infirmary, Glasgow.

2.11.3.3 TOTAL PLASMA ANTIOXIDANT ACTIVITY

A commercial assay, reported to measure the total anti-oxidant capacity of plasma (Randox Ltd., Crumlin, N.Ireland), was also used. This assay relies on the production of the radical cation azinodiethyl-benzthiazoline sulphate^{•+} (ABTS^{•+}) from incubation of ABTS with a peroxidase (metmyoglobin) and hydrogen peroxide. (Miller, Rice-Evans et al. 1993) Production of the radical results in the development of a blue-green colour detectable at 600 nm. Anti-oxidants present in patient plasma inhibit the reaction and (reduce the development of the blue-green colour) to a degree proportional to their concentration. A spectrophotometer is used to quantify the result.

2.11.3.4 PLASMA MALONDIALDEHYDE CONCENTRATION

Malondialdehyde concentration in patient plasma was also considered a measure of oxidative stress (see section 7.2.3.1). (McMurray, Chopra et al. 1993) This is not a measure of antioxidant defence capacity, but is rather a measure of the effect of oxidative stress by assessing lipid peroxidation. Plasma for a thiobarbituric acid-reactive substances (TBARS) assay was collected in sodium heparin tubes and placed on ice. Thiobarbituric acid reacts with malondialdehyde to produce a stable compound that can be quantified using either spectrophotometry or high-performance liquid chromatography (HPLC). The plasma was then cold centrifuged at 1000g for 20 minutes. Aliquots (1.5 mL) were placed in Eppendorf tubes and frozen at -270°C. Assays were run in batches within 2 months of collection by a technician who was blinded to both treatment assignment and collection date.

TBARS were measured by a modification of a colorimetric assay described by Satoh. (Satoh 1978) Briefly, an aliquot of plasma was mixed with 20% trichloroacetic

acid and 0.67% thiobarbituric acid (in a 2 mol/L sodium sulfate solution). After they were agitated in a vortex mixer, the samples were placed in a boiling water bath for 30 minutes and allowed to cool, and butanol was added. The samples were mixed vigorously over a 45 minute period to facilitate extraction of the colored pigments by butanol. Before absorbance of the butanol phase was measured, the sample was centrifuged at 1000g for 10 minutes to ensure purity. Absorbance was measured at 530 nm in comparison to a water blank by use of a spectrophotometer. A malondialdehyde (Sigma Chemical Co; model T-1642) standard curve was shown to be linear up to 20 nmol/mL for this assay. Malondialdehyde standards of 5 and 10 nmol were included with every assay batch, and plasma values of TBARS were expressed in reference to these standards.

2.11.4. CYTOKINE ASSAYS

Dr David Shapiro performed the cytokine assays at the Department of Clinical Biochemistry, Gartnavel General Hospital in Glasgow.

Plasma TNF- α (pg/ml) was measured using a commercially available solid phase enzyme amplified sensitivity immunoassay kit supplied by BioSource Europe SA (Fleurus, Brussels, Belgium). The within assay and between assay coefficients of variation are 3.7% to 5.2% and 8.0% to 9.9% respectively. The minimum detectable concentration and approximate normal range for this assay in healthy subjects are 3 pg/ml and 0 to 20 pg/ml, respectively. All assays were run in duplicate and the average of the two measurements reported.

Plasma sTNFR-2 was measured using a commercially available ELISA test kit (Quantikine) from R&D Systems (Minneapolis, Minnesota). The minimal detectable level is 5pg/ml. The within assay and between assay coefficients of variation are <5.1% and <10% respectively. All assays were run in duplicate and the average of the two measurements reported.

2.11.5. LYMPHOCYTE BETA RECEPTOR DENSITY ASSAY

Dr J.J. Morton in the Department of Medicine and Therapeutics, Western Infirmary, Glasgow, performed these assays in conjunction with Dianne Hillyard.

A 40 ml venous blood sample was collected in an EDTA tube and lymphocytes separated from other blood components by density gradient centrifugation. Briefly, blood was layered over Lymphoprep, centrifuged and the lymphocytes collected from the appropriate band. They were then washed in PBS, collected by low-speed centrifugation and finally resuspended in Tris buffer pH 7.4 at a final concentration of 5 million cells/ml.

The radioligand [^{124}I] iodocyanopindolol (ICYP) (Du Pont) was used for identifying β -adrenergic receptors using the Gilbert modification (Gilbert, Sandoval et al. 1993) of a well established method (Bristow, Minobe et al. 1988). *Total* binding capacity of the lymphocytes was measured by adding 100 μl of phentolamine (an alpha receptor antagonist) solution to 100 μl of lymphocyte preparation and 50 μl of ICYP. *Non-specific beta receptor* binding capacity was measured by adding 100 μl of CGP 12177 (4-(3-tertiary-butylamino-2-hydroxypropoxy)-benzimidazol-2-one hydrochloride, a β_1 and β_2 antagonist with high affinity for the receptors) to 100 μl of lymphocyte preparation and 50 μl of ICYP. Total β -receptor density and K_d were estimated from ICYP saturation curves, with eight increasing concentrations of ICYP between 6.25 and 300 pmol/L.

After mixing and incubation of the preparations for 60 minutes at 37°C, further binding was quenched by placing the preparations on ice. An automated cell harvester was used to harvest the cells on to filter paper disks. The disks were then pressed out

and bound radiolabel quantitated in a gamma counter. An aliquot of the original cell suspension was counted using a Coulter cell counter (automated cell counter), thus allowing calculation of bound radiolabel per cell.

Maximum bound ICYP (B_{\max}) and the ICYP dissociation constant (K_d) were determined by analysis of saturation isotherm data. Non-linear least-squares regression analysis of one form of the Michaelis-Menten equation was performed, where:

$$\text{Bound radioligand} = B_{\max}X / (K_d + X)$$

2.12. ELECTROCARDIOGRAPHIC METHODS

2.12.1. QT DISPERSION

2.12.1.1 MEASUREMENT OF QT DISPERSION

All patients had ECG recordings made at the same paper speed and gain setting (25 mm/sec and 10 mm/mV). We were fortunate in the overall study exclusion criteria in that usual exclusions for QT dispersion studies were already present. A single observer digitised all ECGs using a digitising board (Calcomp, Anaheim, California, USA) connected to an IBM-compatible personal computer and running customised QT dispersion software (Academic Cardiology Unit, Freeman Hospital, Newcastle upon Tyne). QT interval was measured from the beginning of the QRS complex to the end of the T wave, defined as the return to T-P baseline. When U waves were present, the QT interval was measured to the nadir of the curve between the T and U waves. QT intervals were measured in all leads if technically possible. For each lead, two or three consecutive cycles were measured and the arithmetic mean of the QT interval for that lead was used in all future calculations for QT dispersion. The measured values were then expressed as both uncorrected and rate-corrected QT intervals (QT and QTc).

2.12.2. METHODS: SIGNAL-AVERAGED ECG

2.12.2.1 MEASUREMENT OF SIGNAL-AVERAGED ECG

The propensity to ventricular arrhythmias is an important determinant of prognosis in cardiac failure. In several of the large-scale beta-blocker studies, sudden cardiac death (believed to be due to ventricular arrhythmia in most cases) was an end-point that was

particularly susceptible to the influence of beta-blockers.(Chadda, Goldstein et al. 1986; Packer, Bristow et al. 1996; 1999; 1999; Hjalmarson and Fagerberg 2000)

The signal-averaged ECG was initially developed to allow recording of low-amplitude ventricular depolarisations on the surface ECG of myocardial infarct patients who are susceptible to ventricular tachycardia,(Kuchar, Thorburn et al. 1987; Farrell, Bashir et al. 1991) and has since been applied to many different groups of patients.(Gomes, Cain et al. 2001) The link between these ventricular “late potentials” recorded in the SAECD and re-entrant ventricular tachycardia in a wide variety of conditions is well established. (Breithardt, Cain et al. 1991) Viable cells within or surrounding myocardial infarct regions are believed to produce these late potentials which represent slow or delayed conduction and are believed to represent a fixed substrate for re-entrant ventricular tachycardia. Given the susceptibility of CHF patients to ventricular arrhythmia, it was thus of particular interest to determine whether a difference exists between the effect of metoprolol and carvedilol on signal-averaged ECG in this study.

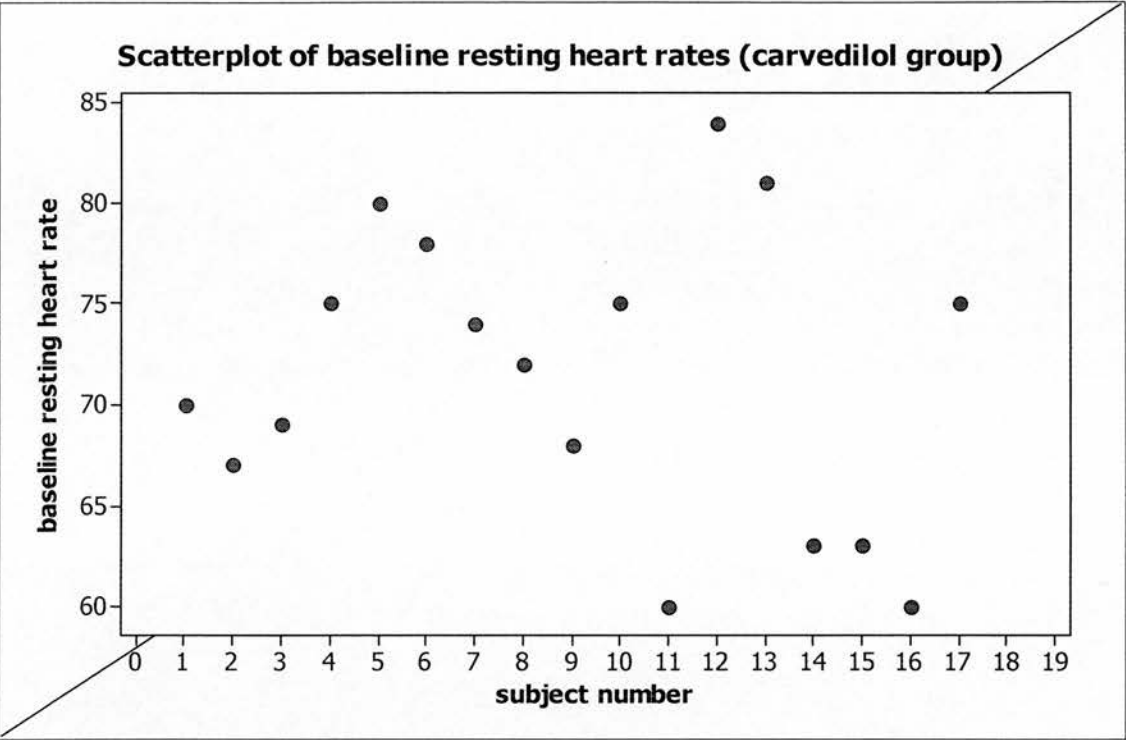
A Megacart ECG machine (Siemens, UK) with signal averaged ECG (SAECD) recording software installed was used to record all SAECDs. SAECDs were recorded immediately after the 12 lead ECG recordings.

CHAPTER 3: STATISTICAL ANALYSIS

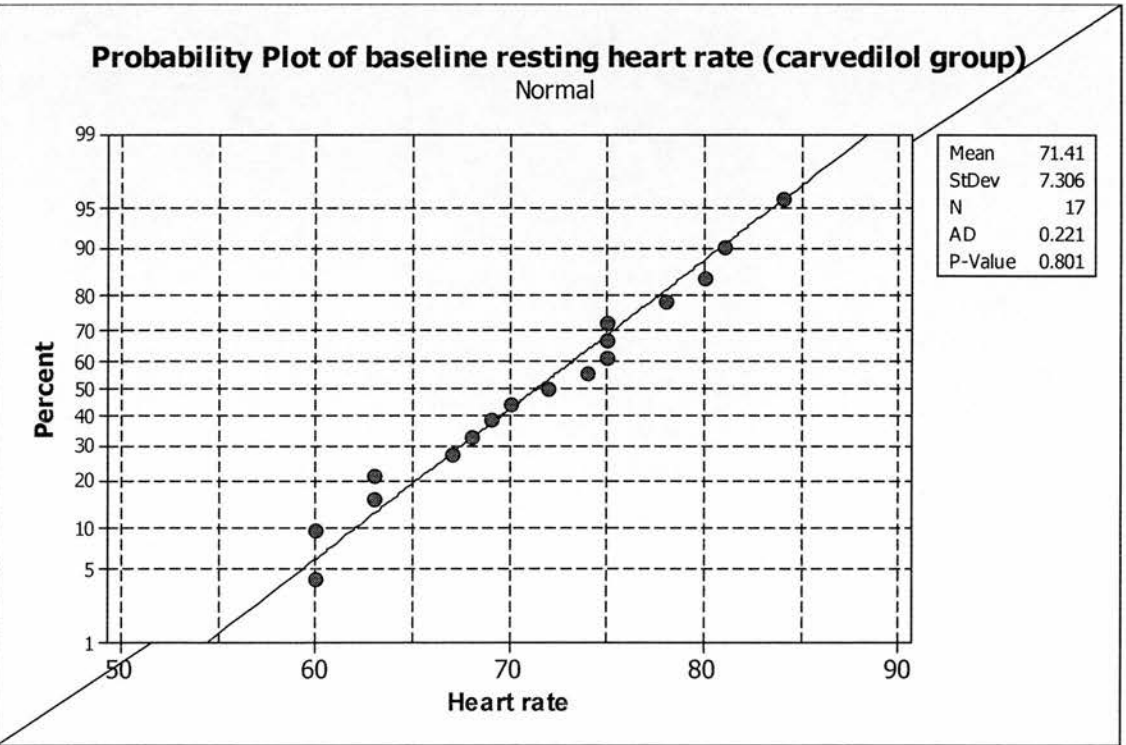
3.1. HAEMODYNAMIC MEASURES

All data was examined using scatterplots and was also subjected to the Anderson-Darling analysis (using Minitab statistical software, Minitab Inc.) to assess whether it was normally distributed. Examples of these graphs are shown below. As the haemodynamic data was all normally distributed, the paired Student's t-test was used to evaluate differences in baseline parameters between a group at the first visit (just before introduction of a beta-blocker) and the same group at the final visit (after 20 weeks of therapy). Comparisons were also made between haemodynamic parameters at the peak dose of the adrenoreceptor agonist administered measured at these two visits. During the analysis, all "washout period" variables (between the two active infusions) were confirmed by paired Student's t-test to have returned to levels not significantly different from the original baseline. Values of $p < 0.05$ were considered significant in all analyses. The data was processed using the Minitab statistical software package (Minitab Inc.) on an IBM-compatible personal computer.

Example scatterplot of normally-distributed data



Example of Anderson-Darling analysis of normally distributed data



3.2. PERIPHERAL ARTERIAL MEASURES

Data from the Chart software package on the PowerMac (points at the start and finish of gradients measuring forearm circumference) were converted to a spreadsheet of data within Microsoft Excel, and then analysed using the Minitab statistical software package (Minitab inc.) on an IBM compatible personal computer.

Each gradient representing forearm swelling of the infused arm was divided by the gradient representing swelling of the non-infused arm, thus producing a ratio at each precise time point when measurements were being made. The mean of five measurable gradient ratios was then used to represent the effect of the agonists on blood flow to the infused forearm at that specific dose level.

Cumulative dose response curves were then constructed of forearm blood flow against individual dose of the individual vasoactive substance. Area under the curve (AUC) was calculated for each dose-response curve. All data was examined using scatterplots and was also subjected to the Anderson-Darling analysis to assess whether it was normally distributed. As the data was indeed normally distributed, the carvedilol group AUCs for all four vasoactive agents at the start of treatment were compared to the AUCs at the end of 20 weeks of treatment using the paired t-test. Similar comparisons were made within the metoprolol group. This is believed to be the most appropriate way of comparing serial measurements of this type.

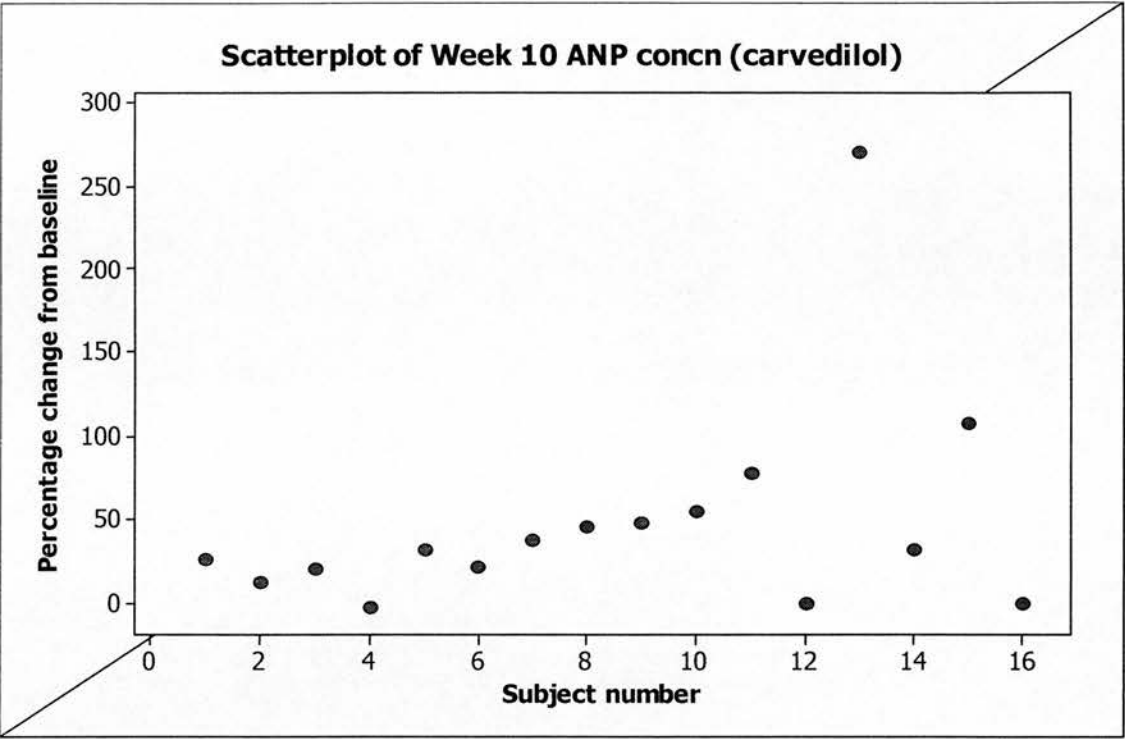
Finally, area under the curve was calculated for ischaemic vasodilatation, and the reactive hyperaemic response of forearm blood flow at the start of the study was compared to that at the end of the study using paired t-test. Data was again analysed

using the Minitab statistical software package (Minitab inc.) on an IBM compatible personal computer.

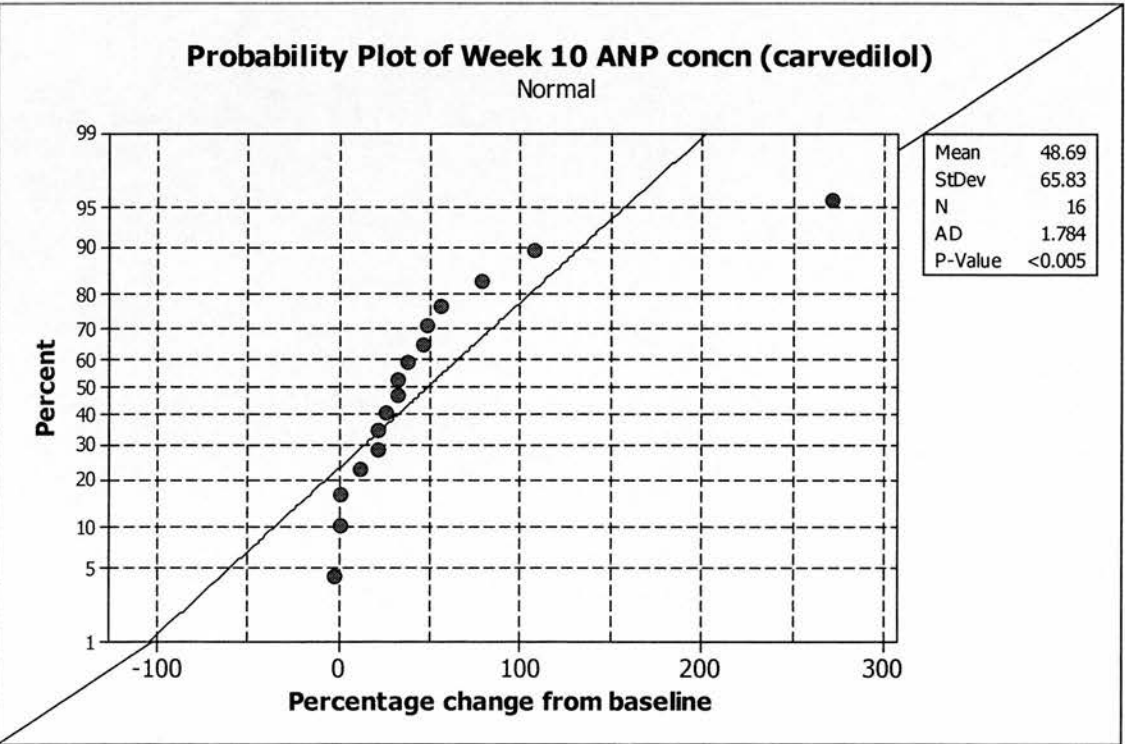
3.3. NEUROHUMORAL MEASURES

Due to the small numbers involved in the study, the means of the two groups (carvedilol-treated and metoprolol-treated patients) were not equal at baseline. Thus all neurohumoral measures are presented as per cent change from baseline. All data was examined using scatterplots and was also subjected to the Anderson-Darling analysis to assess whether it was normally distributed. When the data was confirmed to be normally distributed, paired t-tests were used to compare the results at 10 weeks (end of titration period) and at twenty weeks (end of maintenance period) with baseline measures. When the data was found not to be normally distributed (example shown below), the 1-sample sign test was employed instead. A p-value of less than 0.05 was regarded as significant. Data was analysed using the Minitab statistical software package (Minitab inc.) on an IBM compatible personal computer. Results are presented in graphical form as mean \pm standard error of the mean for parametric data, and as median \pm 95% confidence intervals for non-parametric data.

Example scatterplot of non-parametric data



Example of non-parametric data assessed by Anderson-Darling method



3.4. ANTIOXIDANT MEASURES

Due to the small numbers involved in the study, the means of the two groups (carvedilol-treated and metoprolol-treated patients) were not equal at baseline. Thus all anti-oxidant measures are also presented as per cent change from baseline. All data was examined using scatterplots and was also subjected to the Anderson-Darling analysis to assess whether it was normally distributed. When the data was confirmed to be normally distributed, paired t-tests were used to compare the results at 10 weeks (end of titration period) and at twenty weeks (end of maintenance period) with baseline measures. When the data was found to be non-parametric, the 1-sample sign test was employed instead. A p-value of less than 0.05 was regarded as significant. Data was analysed using the Mintab statistical software package (Minitab inc.) on an IBM compatible personal computer. Results are presented in graphical form as mean \pm standard error of the mean for parametric data, and as median \pm 95% confidence intervals for non-parametric data.

3.5. CYTOKINES MEASURES

All cytokine measures are also presented as per cent change from baseline. Data was analysed using the Mintab statistical software package (Minitab inc.) on an IBM compatible personal computer. All data was examined using scatterplots and was also subjected to the Anderson-Darling analysis to assess whether it was normally distributed. When the data was confirmed to be normally distributed, paired t-tests were used to compare the results at 10 weeks (end of titration period) and at twenty weeks (end of maintenance period) with baseline measures. When the data was found to be non-parametric, the 1-sample sign test was employed instead. A p value of less

than 0.05 was regarded as significant. Results are presented in graphical form as mean \pm standard error of the mean for parametric data, and as median \pm 95% confidence intervals for non-parametric data.

3.6. LYMPHOCYTE BETA RECEPTOR DENSITY

As the mean baseline lymphocyte beta-receptor densities in the two groups were different, it was decided to analyse the data as the percentage change from baseline. Data was entered into an Excel (Microsoft Corporation) spreadsheet. The data was processed using the Minitab statistical software package (Minitab Inc.) on an IBM-compatible personal computer. All data was examined using scatterplots and was also subjected to the Anderson-Darling analysis to assess whether it was normally distributed. For parametrically-distributed data, within group paired student t-tests were performed to look at the effects of the drugs individually over time. When the data was found to be non-parametric, the 1-sample sign test was employed instead. Values of $p < 0.05$ were considered significant in all analyses. Results are presented in graphical form as mean \pm standard error of the mean for parametric data, and as median \pm 95% confidence intervals for non-parametric data.

3.7. QT DISPERSION

As the mean baseline QT dispersion in the two groups were different, it was decided to analyse the data as the percentage change from baseline. Data was entered into an Excel (Microsoft Corporation) spreadsheet. The data was processed using the Minitab statistical software package (Minitab Inc.) on an IBM-compatible personal computer.

All data was examined using scatterplots and was also subjected to the Anderson-Darling analysis to assess whether it was normally distributed. For parametrically-distributed data, within group paired student t-tests were performed to look at the effects of the drugs individually over time. When the data was found to be non-parametric, the 1-sample sign test was employed instead. Values of $p < 0.05$ were considered significant in all analyses. Results are presented in graphical form as mean \pm standard error of the mean for parametric data, and as median \pm 95% confidence intervals for non-parametric data.

3.8. SIGNAL AVERAGED ELECTROCARDIOGRAM

Again, as the mean baseline parameters in the two groups were different, it was decided to analyse the data as the percentage change from baseline. Data was entered into an Excel (Microsoft Corporation) spreadsheet. The data was processed using the Minitab statistical software package (Minitab Inc.) on an IBM-compatible personal computer. All data was examined using scatterplots and was also subjected to the Anderson-Darling analysis to assess whether it was normally distributed. For parametrically-distributed data, within group paired student t-tests were performed to look at the effects of the drugs individually over time. When the data was found to be non-parametric, the 1-sample sign test was employed instead. Values of $p < 0.05$ were considered significant in all analyses. Results are presented in graphical form as mean \pm standard error of the mean for parametric data, and as median \pm 95% confidence intervals for non-parametric data.

CHAPTER 4: HAEMODYNAMIC MEASUREMENTS

4.1. OBJECTIVE

The objective of this component of the study was to compare the haemodynamic effects of treatment with carvedilol and metoprolol in patients with CHF. All haemodynamic parameters were compared at rest and in response to two forms of pharmacological (adrenergic) stress, with a β_1 agonist and with a β_2 agonist. The effects of the two beta-blockers on heart rate and blood pressure were measured, and their influence on cardiac output was assessed using the a bioimpedance monitor (as described in sections 2.9.3, 2.9.4 and 4.2). Measurements were made both early after initiation of beta-blocker therapy and also after chronic therapy (after 20 weeks of drug administration).

Metoprolol is a relatively selective β_1 blocker without vasodilator activity. Carvedilol is a non-selective beta-blocker with vasodilator action mediated via α_1 adrenoreceptor blockade. It has been suggested that the vasodilator action of carvedilol may help offset the reduction in cardiac output anticipated in response to its beta-blocking action, so making the agent less likely to provoke worsening heart failure in CHF patients. With this action there is a risk that the vasodilator action might precipitate symptomatic postural hypotension.

The response of subjects to adrenergic activation (achieved by administration of the relatively specific agonists dobutamine and salbutamol) is of particular interest, as this enables the characterisation of the individual β adrenoreceptor components of adrenergic blockade produced by carvedilol and metoprolol.

4.2. PRINCIPLES OF IMPEDANCE CARDIOGRAPHY

Impedance cardiography employs Ohm's law, where resistance in an alternating current is called impedance (Z) and can be calculated as $Z=V/I$, where V is the voltage and I is the current. The human body is assumed to behave as a "parallel conductor" (the electrical model generally used for the human body), and the assumption is also made that the impedance of thoracic tissue is parallel to that of blood. A sinusoidal current of 50-100 kHz is passed between electrodes on the thorax, and the resultant voltage measured between other electrodes. As current strength is known, the impedance can be calculated.

Other than fluctuations caused by respiration, the impedance of thoracic tissue remains constant. However, impedance changes across the thorax with every heart beat due to alteration in the quantity of blood within the thorax, and this variation in impedance permits estimations of cardiac output to be made.

Several variations in the technique of recording transthoracic signals exist, but one of the most popular in current usage is the eight spot or lateral spot array, where two electrodes are placed at both top and bottom of each side of the thorax. Whilst popular and easier to use than some of the alternative methods, values found for baseline thoracic impedance (necessary for stroke volume calculation) are not comparable between individuals. The "NCCOM" apparatus negotiates this problem by inputting data such as the patient height, weight and gender to the equation and so adjusting calculations as appropriate to the individual.

The impedance signal, its first derivative, and their relationship to the ECG are shown in the diagram (see Figure 4-1). Precise physiological correlates for the different features of the impedance signal shown remain controversial.

Heart cycle-related impedance changes are superimposed on impedance changes caused by respiration. The computer-supported ensemble averaging technique eliminates the effects of respiration on impedance, but at the same time eliminates the possibility of making beat-to-beat measurements of stroke volume. Digital filtering techniques preserve the beat-to-beat variation but are far more complex, in part due to the difficulty in filtering out movement artefact, which can overlap the impedance signal spectrum.

To explain the pulsatile variations in the impedance signal, certain assumptions are made:

1. The impedance of thoracic tissue is parallel to that of intrathoracic blood
2. The resistivity of blood is constant during the cardiac cycle
3. The thorax from the base of the neck to the xiphoid process is a truncated cone which encompasses an elastic cylindrical tube of the same length (the aorta)
4. Electrical current distribution in the truncated cone is homogeneous
5. The maximal change in impedance (dZ/dt_{\max}) multiplied by the left ventricular ejection time is directly proportional to the systolic pulsatile change in aortic blood volume.

That point one is valid is well established. Point 2 is more controversial, but probably makes a very small impact on the overall magnitude of the dZ/dt_{\max} . The third assumption is a clear simplification but alternative models have not yet been validated. The basic thoracic impedance is ultimately linear, related to the inner distance between the voltage detecting electrodes. The fifth assumption is mathematically correct.

The Sramek-Bernstein equation is the most widely used means of calculating the stroke volume, and is the means employed in the commercially available apparatus used in this study: the NCCOM, produced by BoMed medical manufacturing Ltd., Irvine, CA, USA. Stroke volume estimation is based on the following equation:

$$SV = \delta * ((0.17 * H)^3 * (dZ/dt_{\max}) * LVET) / (4.25 * Z_0) \quad , \text{ where:}$$

- 1) Stroke volume is measured in ml
- 2) δ is a weight correction factor, and is affected also by the subject's gender
- 3) L is the distance between the voltage measuring electrodes (cm), and equals about 17% of a subject's height (H)
- 4) V is the volume of the electrical conductor (the truncated cone), and is estimated as $L^3/4.25$
- 5) Z_0 is the basic thoracic impedance (Ω)
- 6) dZ/dt_{\max} is the maximal impedance change (Ω/s)
- 7) LVET is the left ventricular ejection time (s)

Most validation studies found a significant correlation between impedance cardiography and other methods of measuring the stroke volume,(Drazner, Thompson et al. 2002) but the assumptions do not hold under all conditions. Although the exact source of the impedance cardiogram is still unknown, the method is regarded as an accurate means of measuring the stroke volume, and consequently of measuring cardiac output.

4.3. RESULTS

The specific baseline characteristics pertaining to haemodynamic measurements are recorded below (see Table 4-1).

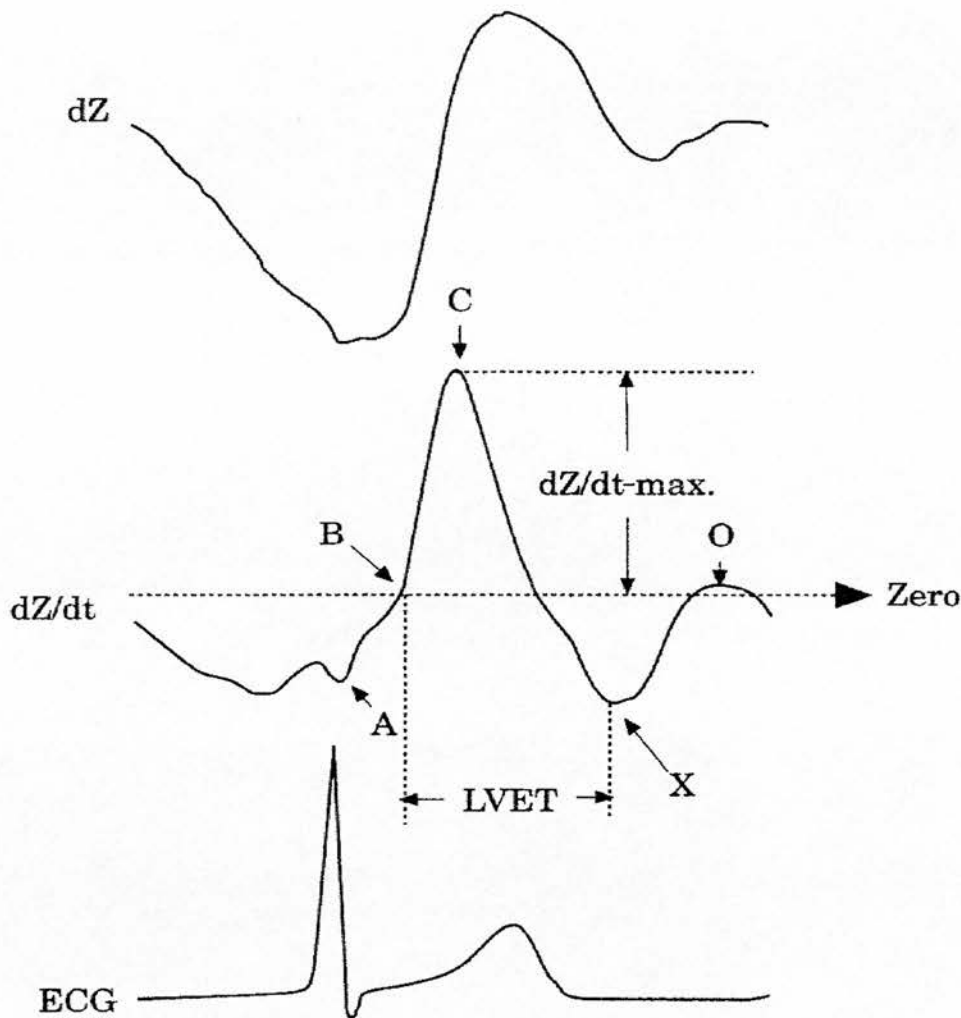
4.3.1. EFFECT OF TREATMENT ON BASELINE VARIABLES

4.3.1.1 RESTING HEART RATE

Seventeen patients in the carvedilol group had a complete set of heart rate data to analyse, and eleven in the metoprolol group. There was a highly significant decrease ($p<0.0001$) in the baseline heart rate of the carvedilol group between the start and the end of the study. There was a highly significant decrease ($p<0.0001$) in the baseline heart rate of the metoprolol group between the start and the end of the study (see Table 4-4).

The degree of resting heart rate reduction in the metoprolol group was slightly greater than in the carvedilol group (mean 14.55 bpm reduction for metoprolol versus 10.12 bpm reduction for carvedilol) but this effect did not reach statistical significance ($p=0.12$). This is illustrated in figure 4-2.

Figure 4-1 : Impedance signal, its first derivative, and their relationship to ECG



The middle trace shows the variation in the impedance signal over time.

- A = downward deflection due to contraction of the atria
- B = start of left ventricle ejection
- C = the major upward deflection occurring during systole
- X = closure of the aortic valve
- O = diastolic upward deflection
- LVET = left ventricular ejection time (s)
- dZ/dt_{max} = maximal impedance change during systole

4.3.1.2 RESTING SYSTOLIC BLOOD PRESSURE

Baseline systolic blood pressure readings were not significantly altered in the carvedilol group or in the metoprolol group, comparing study start with study end (see Table 4-4).

4.3.1.3 RESTING DIASTOLIC BLOOD PRESSURE

Baseline diastolic blood pressure readings were not significantly altered in the carvedilol group or in the metoprolol group, comparing study start with study end (see Table 4-4).

4.3.1.4 RESTING CARDIAC OUTPUT

No significant change over the course of the study was seen in baseline cardiac output in either the carvedilol group or the metoprolol group (see Table 4-4).

4.3.1.5 RESTING STROKE VOLUME

There was no significant difference in stroke volume in the carvedilol group nor in the metoprolol group between the start and the end of the study (see Table 4-4).

4.3.2. EFFECT OF TREATMENT ON RESPONSE TO DOBUTAMINE

4.3.2.1 HEART RATE

There was no significant difference between the start and end of the study in the heart rate recorded during infusion of the highest dose of dobutamine to the carvedilol group i.e. the increase in heart rate caused by dobutamine was not blunted despite 20

weeks of treatment with carvedilol. There was a significant decrease ($p=0.009$) between the start and the end of the study in the heart rate recorded in the metoprolol group during infusion of the highest dose of dobutamine (Figure 4-3 and Table 4-5).

4.3.2.2 SYSTOLIC BLOOD PRESSURE

There was no significant effect of long-term treatment on the systolic blood pressure achieved during the highest dose dobutamine infusion in either the carvedilol or the metoprolol group (see Table 4-5 and Figure 4-4).

4.3.2.3 DIASTOLIC BLOOD PRESSURE

There was no significant effect of long-term treatment on the diastolic blood pressure achieved during the highest dose dobutamine infusion in either the carvedilol or the metoprolol group (see Table 4-5 and Figure 4-5).

4.3.2.4 CARDIAC OUTPUT

In the carvedilol group, there was a significant ($p=0.03$) decrease of cardiac output between that measured during infusion of the highest dose of dobutamine at the start of the study as compared with the same dose of dobutamine at the end of the study.

There was no significant difference in cardiac output in the metoprolol group between study start and end (see Table 4-5 and Figure 4-6).

4.3.2.5 STROKE VOLUME

In the carvedilol group, there was a significant ($p=0.047$) decrease between the start and the end of the study in the stroke volume measured during infusion of the highest

dose of dobutamine (see Table 4-5 and Figure 4-7). There was no significant effect on the stroke volume of the metoprolol group.

4.3.3. EFFECT OF TREATMENT ON RESPONSE TO SALBUTAMOL

4.3.3.1 HEART RATE

The decrease, between study start and end, in mean heart rate during infusion of the highest dose of salbutamol in the carvedilol group (mean decrease of 4.75 beats per minute) just failed to reach significance ($p=0.051$). The equivalent decrease (10.27 bpm) in the metoprolol group was highly significant ($p=0.007$) (see Table 4-6 and Figure 4-8).

4.3.3.2 SYSTOLIC BLOOD PRESSURE

There was no significant effect of long-term treatment on the systolic blood pressure achieved during the highest dose salbutamol infusion in either the carvedilol or the metoprolol group (see Table 4-6 and Figure 4-9).

4.3.3.3 DIASTOLIC BLOOD PRESSURE

There was no significant effect of long-term treatment on the diastolic blood pressure achieved during the highest dose salbutamol infusion in either the carvedilol or the metoprolol group (see Table 4-6 and Figure 4-10).

4.3.3.4 CARDIAC OUTPUT

There was no significant difference between the start and end of the study in the cardiac output recorded during infusion of the highest dose of salbutamol in either group (see Table 4-6 and Figure 4-11).

4.3.3.5 STROKE VOLUME

There was no significant difference between the start and end of the study in the stroke volume measured during infusion of the highest dose of salbutamol in either group (see Figure 4-12 and Table 4-6).

4.4. DISCUSSION

In this study, both drugs produced a significant decrease in resting heart rate after 20 weeks of treatment as might be expected. There was no distinct difference between the agents in the *degree* of reduction in resting heart rate. There was no statistically significant decrease in either resting systolic or resting diastolic blood pressure in either carvedilol or metoprolol groups over the same period. There was also no statistically significant change in resting cardiac output or stroke volume in either group.

Differences appear when the responses to peak dose dobutamine and salbutamol are analysed. The heart rate response of the carvedilol group to peak dose dobutamine was surprisingly *not significantly attenuated* after 20 weeks treatment. The carvedilol effect on heart rate response to salbutamol only just failed to reach statistical significance ($p=0.051$). Metoprolol, on the other hand, produced significant

attenuation in the heart rate response to both dobutamine and salbutamol after this period of treatment. Thus metoprolol produced more complete beta-blockade in response to beta agonism than carvedilol.

Blood pressure responses to the agents were not significantly different from baseline. In the carvedilol group, there was actually a significant decrease in both cardiac output and in stroke volume in response to peak dose dobutamine. There was no such response seen during peak dose salbutamol infusion. In the other group, no significant changes in cardiac output or in stroke volume were produced after chronic therapy with metoprolol.

4.4.1. HEART RATE

The patients enrolled in this study had a considerably lower baseline resting heart rate than those enrolled in studies such as COMET (see Table 4.2). Despite this, the magnitude of heart rate reduction, expressed as percentage reduction from baseline, is similar in this study to the other studies listed which also used metoprolol and carvedilol (Table 4.2). Indeed, the reduction in resting heart rate with metoprolol is if anything slightly greater than that seen in large studies.

One possible explanation for the lower baseline heart rate in the patients in this study is that the use of beta-blockers had been permitted prior to study entry. However, review of the demographic data (see Table 2-4) reveals that only 11% of carvedilol-treated patients had been on beta-blockers prior to study entry, and only 40% of metoprolol-treated patients. In addition, these agents were withdrawn a few days prior

to baseline assessments, so if anything an increased resting heart rate might have been expected.

Another obvious possibility is that the patients were clinically better than those enrolled in the large studies. All patients had good clinical and echocardiographic evidence to support the diagnosis of chronic heart failure, but it may be that they were suffering from a less severe degree of CHF, or were better compensated.

A final factor may be the care taken in this study to ensure the patients were assessed after a period of rest. The intention of this was to permit neurohumoral measures rather than heart rate to settle to baseline, but it would obviously have had an effect on heart rate also.

Both carvedilol and metoprolol caused a highly significant decrease in baseline heart rate between the start and the end of the study (see Table 4-4). These actions would of course be entirely anticipated when administering beta-blockers. The magnitude of effect on resting heart rate of chronic treatment with the individual beta-blockers is very similar, suggesting the doses used were a reasonable comparison. The effect of metoprolol on the resting heart rate is slightly greater than that of carvedilol, in both absolute terms and in percentage reduction from baseline (see Table 4.2), but this effect is far from being statistically significant ($p=0.12$). The metoprolol action is somewhat greater, and the carvedilol action somewhat less, than other large studies involving these agents (see Table 4.2 for figures and for comparison with other studies).

Metoprolol also seemed to produce a more profound degree of beta blockade in response to beta agonism. There is some support for this finding in the literature.

(Stoschitzky, Koshucharova et al. 2001) One possible explanation for the apparently greater beta blocking effect of metoprolol compared to carvedilol would be differences in the timing of onset of the maximal effect of the two beta blockers. Metoprolol, most particularly the short-acting form employed in our study, might be expected to reach maximal effect more quickly, but would obviously not have such a prolonged effect. In fact, both agents actually reach maximum plasma concentrations within a similar time frame of one to two hours.(Luzier, Killian et al. 1999; Dulin and Abraham 2004) As the timing of heart rate measures in our study was 3 hours after dosing, both drugs were at peak effect and if anything the effect of metoprolol (shorter-acting) might have been expected to have been waning.

A possible explanation for a differential effect of the two drugs on heart rate is an unusually high incidence of a specific beta-receptor polymorphism in one of our treatment groups (not present in the other) resulting in different heart rate responses. Such a dichotomy of response to beta-blockers has been noted in the MERIT-HF study, in that instance resulting in similar degrees of heart rate reduction with different doses (and different resultant plasma concentrations) of metoprolol.(Wikstrand, Hjalmarson et al. 2002) About one third of patients in this re-analysis had a marked heart rate response to a low dose of metoprolol. Despite the low dose of active drug, these same patients had a similar magnitude of heart rate reduction and of reduction in mortality as those who tolerated a high dose of metoprolol. It is conceivable that more patients with a small heart rate response to beta-blocker were present in our metoprolol group. This effect would of course be more marked in a study with small numbers of patients.

We do not appear to have used a dose of metoprolol which is too large. The MERIT-HF study had a target daily dose of metoprolol, albeit in a slow-release preparation, of 200 mg (as opposed to 100 mg in this study) and the achieved dose of metoprolol succinate (slow release) was 159mg per day. This equates to 106 mg of metoprolol tartrate per day when the lower bioavailability of the succinate form is taken into account.(Dargie 2003) Some other large-scale carvedilol studies have employed the same target dose for carvedilol as we did (25 mg BD).

It is possible that the vasodilating action of carvedilol produces a reflex tachycardia. As both dobutamine and salbutamol are peripheral vasodilators, the combined action of either drug plus that of carvedilol might result in a reflex tachycardia of sufficient magnitude to counteract some of the beta-blocker induced heart rate reduction.

With the publication of the COMET trial results,(Poole-Wilson, Swedberg et al. 2003) data from a large number of CHF patients undergoing treatment with these two beta blockers has become available. In COMET, the mean daily dose of carvedilol in the maintenance phase of the study was 41.8 mg a day. In the metoprolol group, the mean daily dose was 85 mg a day. These dosages compare very closely with the doses used in this study (44.4 mg/day carvedilol and 91.7 mg/day metoprolol). However, as a consequence of criticisms about the relative degrees of beta blockade in the two groups, the COMET authors have been at pains to stress that the magnitude of heart rate reduction with both agents was very similar. We also made this observation in our study (see Table 4.2).

Carvedilol did not reduce the increase in heart rate seen in response to dobutamine even after 20 weeks of treatment. Carvedilol is less specific at the β_1 receptor than metoprolol, and thus would not necessarily be expected to have such a potent effect as

metoprolol. Other observers contradict our findings with carvedilol in response to dobutamine.(Metra, Nodari et al. 2002; Bollano, Tang et al. 2003) In both of these studies, carvedilol had a profound effect to suppress the heart rate increase normally seen with dobutamine, even though the dose of dobutamine employed in both studies was greater: Metra et al. used 20mcg/kg/min whilst Bollano et al. used 15mcg/kg/min. The dose used in our study was 10mcg/kg/min at peak. Bollano certainly used a very large dose of carvedilol (mean 85mg compared to our mean 44.4mg per day) which might explain the findings of that study.(Bollano, Tang et al. 2003) However, Metra used a very similar dose to that employed by us (mean 43mg per day).(Metra, Nodari et al. 2002) It is difficult to reconcile this discrepancy.

The carvedilol effect to reduce the heart rate response to *salbutamol* was also less than that of metoprolol: note that the mean reduction with carvedilol was 4.75 bpm, $p=0.051$, whilst the mean reduction with metoprolol was 10.27 bpm, $p=0.007$. This is surprising as carvedilol is definitely active at the β_2 receptor and more so than metoprolol. This might suggest that metoprolol has important β_2 receptor actions as well as the anticipated β_1 receptor action. In fact, it is clear that metoprolol is by no means entirely specific to β_1 receptors (see Table 4-3).

Based on the results of previous very large-scale studies, the doses of metoprolol and carvedilol we employed would in fact be expected to produce similar degrees of heart rate reduction, and indeed were used in the COMET trial. This issue is discussed further in section 11.2, and is also illustrated in Table 4.2.

Metoprolol is a selective antagonist of adrenergic β_1 receptors whereas carvedilol is a non-selective beta-blocker with additional α_1 blocking effects. Both drugs are used as

racemates in clinical practice, consisting of equal amounts of (R)- and (S)-enantiomers. The beta blocking effects of both drugs reside in the (S)-enantiomers, but both (R) and (S) enantiomers of carvedilol act as α -blockers. Consequently, the metoprolol used clinically consists of 50% beta-blocker and 50% of a compound, the (R)-enantiomer, with no adrenergic activity. Carvedilol consists of 50% of an antagonist at α_1 and β receptors, (S)-carvedilol, and 50% of a pure α_1 blocker, (R)-carvedilol.(Stoschitzky, Koshucharova et al. 2001)

If one accepts that metoprolol is a “classic” beta-blocker with relative specificity for the β_1 receptor subtype, there are in fact three possible explanations for the lesser effect of carvedilol on beta agonist-induced increase in heart rate:

1. Insufficient beta blockade produced by carvedilol in clinical practice.
2. Intrinsic sympathomimetic activity of (R, S)-carvedilol.
3. Reflex activation of sympathetic tone caused by the α_1 blocking properties of both (R) and (S) carvedilol.

This last possibility is the most likely. In support of this theory, optically pure carvedilol actually *increases* resting heart rate in healthy individuals, supporting the third hypothesis.

As mentioned above in the results section, our findings are in agreement with at least one other study,(Stoschitzky, Koshucharova et al. 2001) though it must be stressed that the study in question was on normal individuals rather than in patients with CHF.

4.4.2. BLOOD PRESSURE

One of the most surprising aspects of the effects of both agents on blood pressure is that resting blood pressure is not significantly affected by treatment with beta-blockers. There is slight reduction in both systolic and diastolic blood pressure with metoprolol but a slight increase with carvedilol (see Table 4-4). The anticipated antihypertensive action of both drugs was not evident in this study. As no obvious blood pressure drop was detectable with carvedilol, concerns previously voiced about its vasodilating properties having the potential to cause postural hypotension seem unfounded in our hands.

4.4.3. CARDIAC OUTPUT AND STROKE VOLUME

The patients enrolled in this study had baseline left ventricular ejection fractions, cardiac outputs and stroke volumes that were not significantly different from each other (see Table 4-1). Carvedilol was much more effective than metoprolol at suppressing the increase in cardiac output and stroke volume normally seen in response to dobutamine (see Figure 4-6, Figure 4-7, Figure 4-11 and Figure 4-12). Metoprolol tends to decrease stroke volume at rest, and this effect just fails to reach significance ($p=0.073$). It would appear that carvedilol, presumably through its broad anti-adrenergic action, acts to protect the heart from increased workload. If the neurohumoral hypothesis were taken as the paradigm, this action would be beneficial.

Previous comparisons of the haemodynamic actions of carvedilol and metoprolol have focused either on hypertensive patients employing the parameters used in our study, (Weber, Bohmeke et al. 1996; Weber, Bohmeke et al. 1998) or on CHF patients but

using parameters such as left ventricular ejection fraction (Sanderson, Chan et al. 1999) or left ventricular stroke volume as means of assessing haemodynamic differences. (Metra, Giubbini et al. 2000) The most extensive publication detailing the comparative effects of carvedilol and metoprolol on left ventricular ejection fraction is a meta-analysis. (Packer, Antonopoulos et al. 2001) In this paper, 19 CHF trials of carvedilol or metoprolol were amalgamated. All were randomized and controlled and all assessed LVEF before and after treatment. The findings were that carvedilol seemed consistently to increase LVEF more than metoprolol. We deliberately did not reassess the LVEF in our small groups after treatment, as it unlikely that a significant difference could have been demonstrated due to the small numbers.

In hypertension, it has been shown that peripheral vascular resistance tends to be increased and cardiac output decreased with “conventional” beta blockers, particularly in the short term. In previous comparisons of metoprolol and carvedilol in hypertension, the mechanisms by which blood pressure reduction was achieved was found to differ between the two drugs. Metoprolol reduces cardiac output, and there is a substantial increase in systemic vascular resistance due to reflex stimulation of α -adrenoreceptors. This increase in vascular resistance persisted for the duration of the studies (4 weeks in both studies). (Weber, Bohmeke et al. 1996; Weber, Bohmeke et al. 1998)

In these hypertension studies the reduction in blood pressure with carvedilol was achieved without a reduction in cardiac output due to α -adrenoreceptor mediated vasodilatation of systemic resistance vessels. Normally a sympathetically mediated reflex tachycardia would accompany this phenomenon, but this was prevented apparently due to the broad beta blocking action of this agent. Interestingly, the

peripheral vasodilating action was seen to be somewhat attenuated at 4 weeks, in keeping with the usual observation that haemodynamic tolerance develops to persistent α -adrenoreceptor blockade. More recently, other authors have confirmed the loss of the α -blocking action of carvedilol after 6 months in CHF.(Hryniewicz, Androne et al. 2003)

In CHF, two studies compare the haemodynamic responses of carvedilol and metoprolol-treated patients to beta agonists. In one study dobutamine was used as part of a dobutamine stress echo protocol.(Bollano, Tang et al. 2003) This single blind cross-over study in ten patients found that dobutamine produced an increase in cardiac output in metoprolol-treated patients that was due to an increase in heart rate: the heart rate increase is accompanied by a very comparable cardiac output increase, as assessed echocardiographically. The heart rate increase occurred in the metoprolol group but not in the carvedilol group. This finding would seem to be at odds with our data. The finding is all the more surprising as the target dose of metoprolol succinate was actually 100 mg BD. A mean dose of 160mg of metoprolol succinate per day was achieved (equivalent to about 107 mg metoprolol tartrate) and would obviously have been expected to produce a greater degree of beta blockade than we achieved, abolishing both heart rate and cardiac output increases. The dose of carvedilol used in the above study (Bollano, Tang et al. 2003) was almost twice that which we used (85mg per day compared to 44.4mg). This very large dose of carvedilol probably explains the marked effect of carvedilol to suppress increase in heart rate and cardiac output as compared with our study. In this respect the findings agree with ours, indicating a marked effect of carvedilol to inhibit the agonist-induced increase in cardiac output.

The second study where cardiac output in response to inotropes has been compared in patients treated with carvedilol and metoprolol also does not compare well with our data. (Metra, Nodari et al. 2002) Here, a very comparable dose of carvedilol was used (43mg per day) and a higher dose of metoprolol. The effect of carvedilol was again very much to suppress any heart rate or stroke volume index increases with dobutamine (as assessed by invasive haemodynamic monitoring). Despite employing a larger dose of metoprolol (129mg per day) the metoprolol did nothing to suppress heart rate and stroke volume increases with dobutamine. This finding is clearly at odds with our results.

4.5. CONCLUSION

This study involved a small number of patients, so there was a large variance for some measures making it impossible to be certain that there is no difference between the groups in some measures. It is also possible some of the apparently statistically significant changes shown are spurious.

In our study, carvedilol and metoprolol at the doses used have very similar effects on resting heart rate, and these actions are comparable to larger studies. It is notable, however, that chronic treatment with carvedilol at the doses employed in our study permits a greater increase in heart rate in response to adrenergic activation than metoprolol. Chronic metoprolol therapy in our hands exerts a more profound beta blocking action with regard to heart rate response to adrenergic activation, even (surprisingly) in response to salbutamol. Thus in pure heart rate terms, carvedilol would appear to be the less effective agent. Possible explanations for this are given above and the issue is also examined in sections 11.2 and 11.3.2.

It is of course already clear that the beneficial clinical effect of both carvedilol and metoprolol in CHF is of a *similar* magnitude. It may be that something other than pure heart rate reduction is occurring to produce the beneficial results seen with carvedilol. This is a finding of intense interest in terms of dissecting the differences between the two agents.

Carvedilol certainly suppresses the normal increase in cardiac output and in stroke volume caused by adrenergic stimulation. In this way, carvedilol seems more effective at preventing increased cardiac workload in CHF in times of stress. This action is supported by data from other studies.(Metra, Nodari et al. 2002; Bollano, Tang et al. 2003)

Table 4-1: Baseline haemodynamic measures

	Carvedilol n=17	s.d.	Metoprolol n=11	s.d.	Statistical difference
Heart Rate (bpm)	71.4	7.31	69.2	11.00	NS (p=0.562)
Systolic Blood Pressure (mm Hg)	112.81	16.69	117.82	20.31	NS (p=0.508)
Diastolic Blood Pressure (mm Hg)	62.12	6.77	66.73	8.52	NS (p=0.146)
Cardiac Output (l/min)	4.44	1.19	3.70	1.19	NS (p=0.125)
Stroke Volume (mls)	70.50	21.94	59.27	16.24	NS (p=0.140)
Left ventricular ejection fraction (%)	27.8	7.3	30.5	7.9	NS (p=0.247)

Table 4.2 Heart rate effects compared to other studies

	<i>Equivalent dose of metoprolol</i>	Reduction in mean HR in BPM (%)	Base HR	HR reduction BPM/mg	Dose of carvedilol	Reduction in mean HR in BPM (%)	Base HR	HR reduction BPM/mg
MDC	108	15 (16.7%)	90	0.139	-	-	-	-
USC	-	-	-	-	45	13 (15.5%)	84	0.289
MERIT- HF	106	14 (17.0%)	82.4	0.132	-	-	-	-
COMET	85	11.7 (14.4%)	81	0.138	41.8	13.3 (16.4%)	81	0.318
MD	91.7	14.54 (21.0%)	69.2	0.159	44.1	10.1 (14.1%)	71.4	0.229

Key to table 4.2: HR=heart rate; BPM=beats per minute; MDC=Metoprolol in Dilated Cardiomyopathy study; USC=United States Carvedilol study; MERIT-HF= Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure; COMET=Carvedilol or Metoprolol European Trial; MD=thesis data

Table 4-3 : Relative specificity of various beta blockers at β_1/β_2 receptors

Class		K(β_1)	K(β_2)	β_1/β_2 selectivity	K(α_1)	β_1/α_1
		nmol/L	nmol/L		nmol/L	selectivity
Nonselective	Propranolol	4.1	8.5	2.1	-	-
Selective β_1	Metoprolol	45	3345	74	-	-
	Bisoprolol	121	14390	119	-	-
β_1 blocker/ vasodilator	Carvedilol	4.0	29	7.3	9.4	2.4

Table 4-4: Effects of treatment on resting haemodynamic measures

Treatment comparison	n	Mean	s.d.	s.e.	95% CI	P value
Carvedilol: heart rate on Day 0 vs final day	17	-10.12	6.14	1.49	6.96- 13.28	<0.0001
Metoprolol : heart rate on Day 0 vs final day	11	-14.55	7.53	2.7	9.49-19.60	<0.0001
Carvedilol : systolic BP on Day 0 vs final day	17	2.44	11.91	2.98	-3.91-8.78	0.426
Metoprolol : systolic BP on Day 0 vs final day	11	-3.55	17.09	5.15	-15.03-7.94	0.507
Carvedilol : diastolic BP on Day 0 vs final day	17	0.69	4.92	1.23	-1.94-3.31	0.585
Metoprolol : diastolic BP on Day 0 vs final day	11	-1.27	8.00	2.41	-6.65-4.10	0.609
Carvedilol : cardiac output on Day 0 vs final day	17	0.206	1.000	0.250	-0.326-0.739	0.422
Metoprolol : cardiac output on Day 0 vs final day	11	0.218	0.703	0.212	-0.254-0.690	0.327
Carvedilol : stroke volume on Day 0 vs final day	17	-0.88	16.48	4.12	-9.66-7.91	0.835
Metoprolol : stroke volume on Day 0 vs final day	11	-7.00	11.58	3.49	-14.78-0.78	0.073

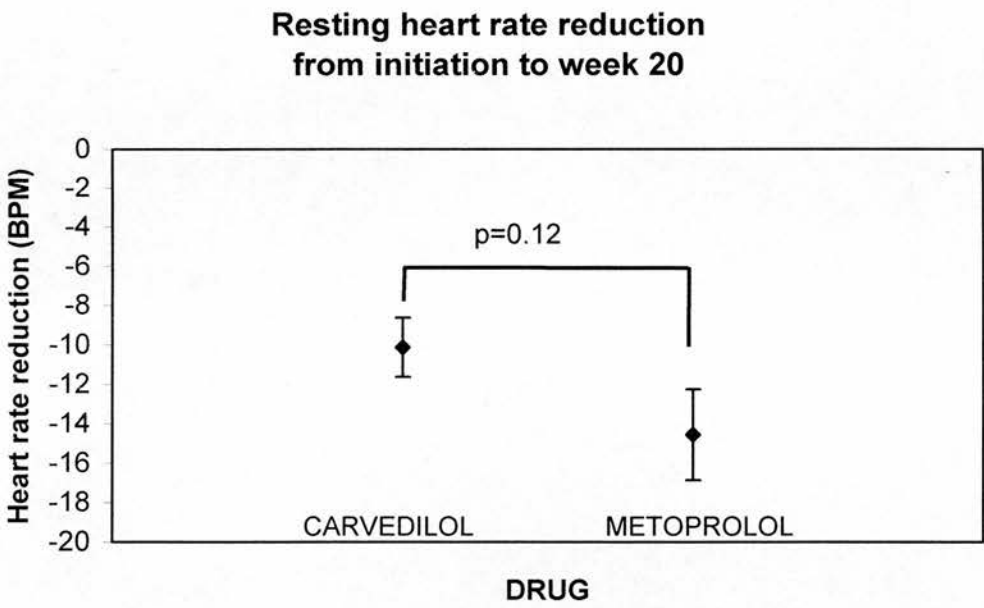
Table 4-5 : Effects of treatment on response of haemodynamic measures to peak dose Dobutamine

Treatment comparison	n	Mean	s.d.	s.e.	95% CI	P value
Carvedilol : heart rate on Day 0 vs final day	17	-1.75	8.27	2.07	2.65-6.15	0.410
Metoprolol : heart rate on Day 0 vs final day	11	-10.64	10.94	3.30	3.29-17.99	0.009
Carvedilol : systolic BP on Day 0 vs final day	17	-4.75	24.66	6.17	-17.89-8.39	0.453
Metoprolol : systolic BP on Day 0 vs final day	11	-4.73	16.29	4.91	-15.67-6.22	0.359
Carvedilol : diastolic BP on Day 0 vs final day	17	0.56	11.94	2.99	-5.80-6.93	0.853
Metoprolol : diastolic BP on Day 0 vs final day	11	-0.91	10.73	3.23	-8.12-6.30	0.784
Carvedilol : cardiac output on Day 0 vs final day	17	0.650	1.082	0.270	0.073-1.227	0.030
Metoprolol : cardiac output on Day 0 vs final day	11	-0.136	0.507	0.153	-0.477-0.204	0.393
Carvedilol : stroke volume on Day 0 vs final day	17	10.19	18.88	4.72	0.13-20.25	0.047
Metoprolol : stroke volume on Day 0 vs final day	11	-7.91	15.08	4.55	-18.04-2.22	0.113

Table 4-6 : Effects of treatment on response to peak dose Salbutamol

Treatment comparison	n	Mean	s.d.	s.e.	95% CI	P value
Carvedilol : heart rate on Day 0 vs final day	17	-4.75	8.96	2.24	-0.03-9.53	0.051
Metoprolol : heart rate on Day 0 vs final day	11	-10.27	10.07	3.04	3.51-17.04	0.007
Carvedilol : systolic BP on Day 0 vs final day	17	-4.00	18.63	4.66	-13.93-5.93	0.404
Metoprolol : systolic BP on Day 0 vs final day	11	-1.55	17.43	5.26	-13.26-10.17	0.775
Carvedilol : diastolic BP on Day 0 vs final day	17	-2.56	10.59	2.65	-8.21-3.08	0.348
Metoprolol : diastolic BP on Day 0 vs final day	11	-0.91	4.78	1.44	-4.12-2.31	0.543
Carvedilol : cardiac output on Day 0 vs final day	17	0.425	1.002	0.250	-0.109-0.959	0.110
Metoprolol : cardiac output on Day 0 vs final day	11	0.127	0.728	0.220	-0.362-0.616	0.575
Carvedilol : stroke volume on Day 0 vs final day	17	1.94	13.38	3.34	-5.19-9.07	0.571
Metoprolol : stroke volume on Day 0 vs final day	11	-6.27	11.98	3.61	-14.32-1.78	0.113

Figure 4-2



Key for Figures 4-3 to 4-12:

- B= baseline (prior to infusion of drug)
- 1= first level of infused drug
- 2= second level of infused drug
- 3= third level of infused drug
- DOSE indicates level of infused drug
- C day 0= first day of treatment with carvedilol/first prolonged study day
- C final= final prolonged study day (carvedilol-treated patient)
- M day 0= first day of treatment with metoprolol/first prolonged study day
- M final= final prolonged study day (metoprolol-treated patient)

Figure 4-3 (see key above)

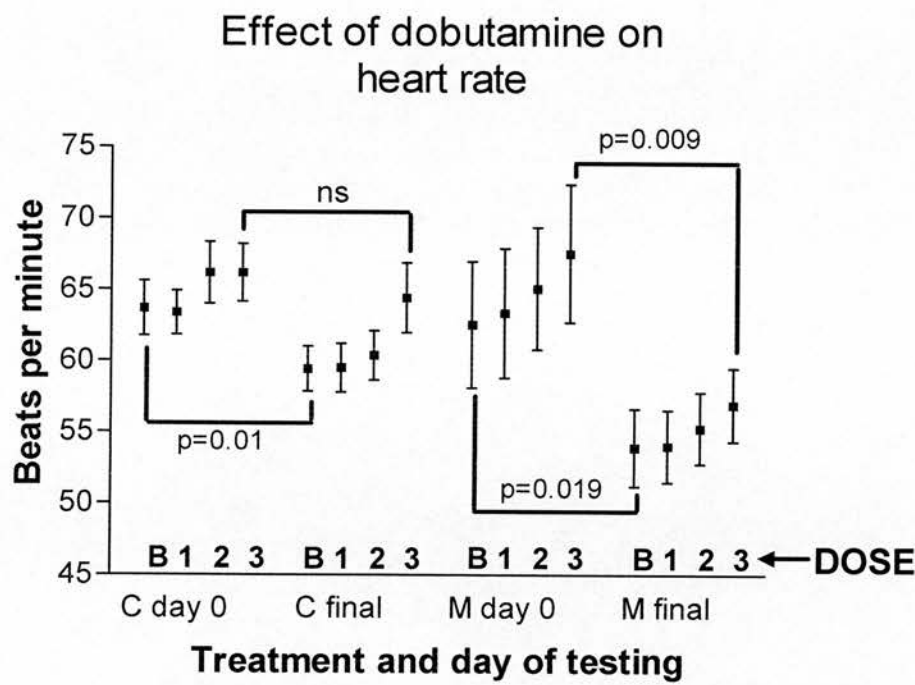


Figure 4-4 (see key above)

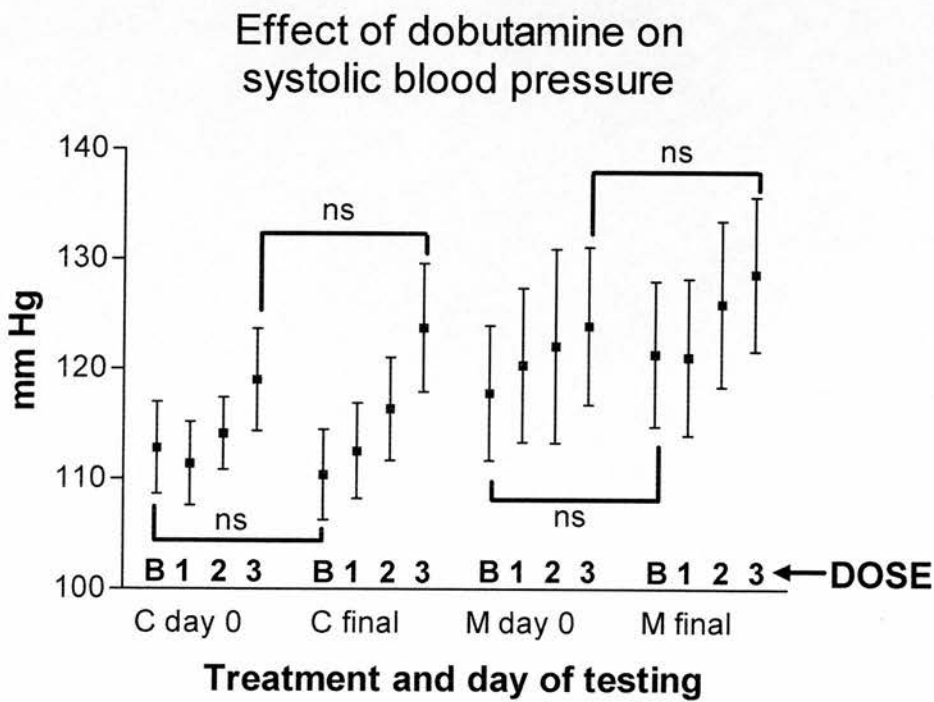


Figure 4-5 (see key above)

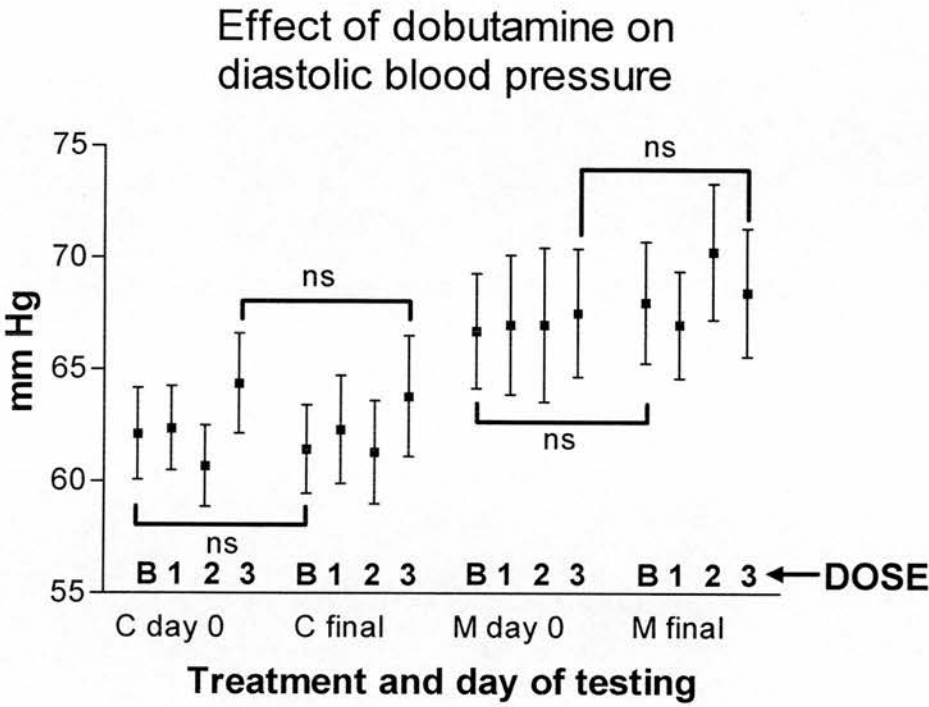


Figure 4-6 (see key above)

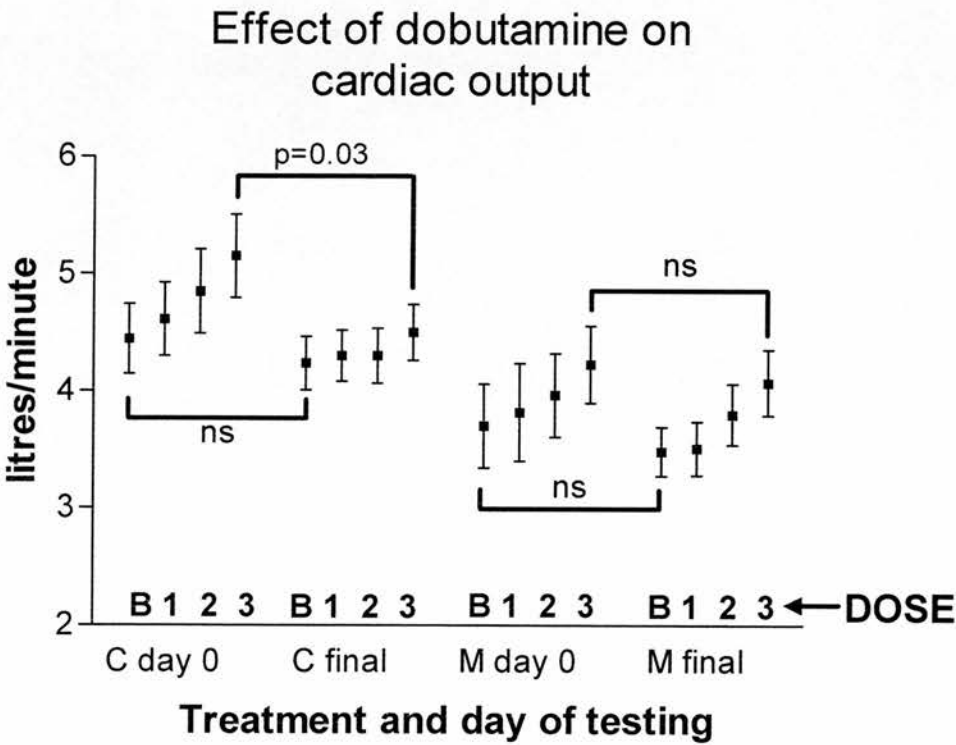


Figure 4-7 (see key above)

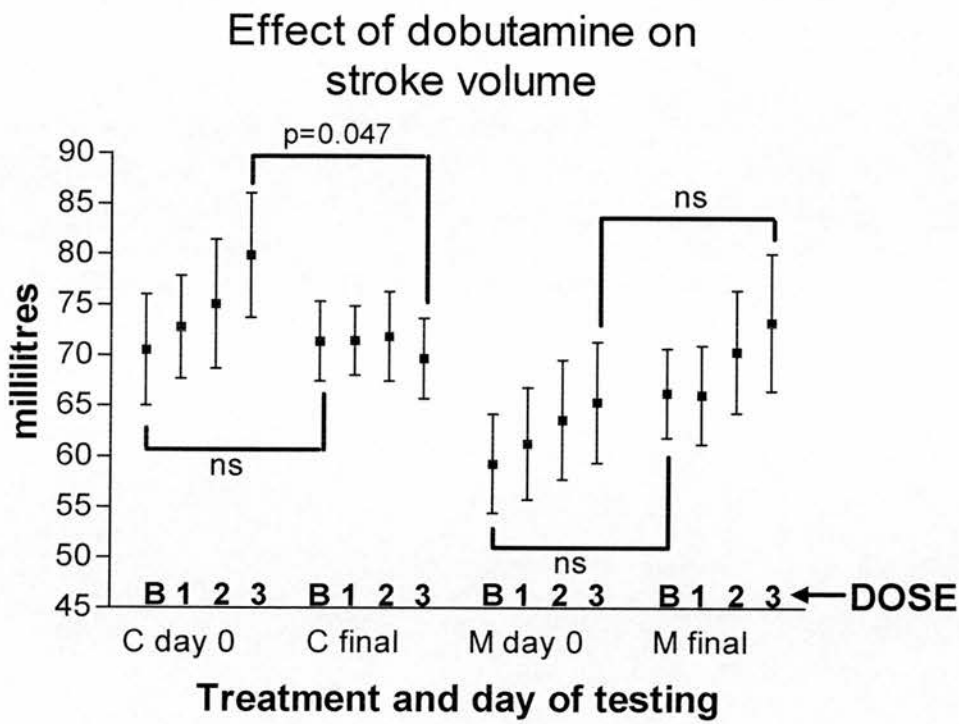
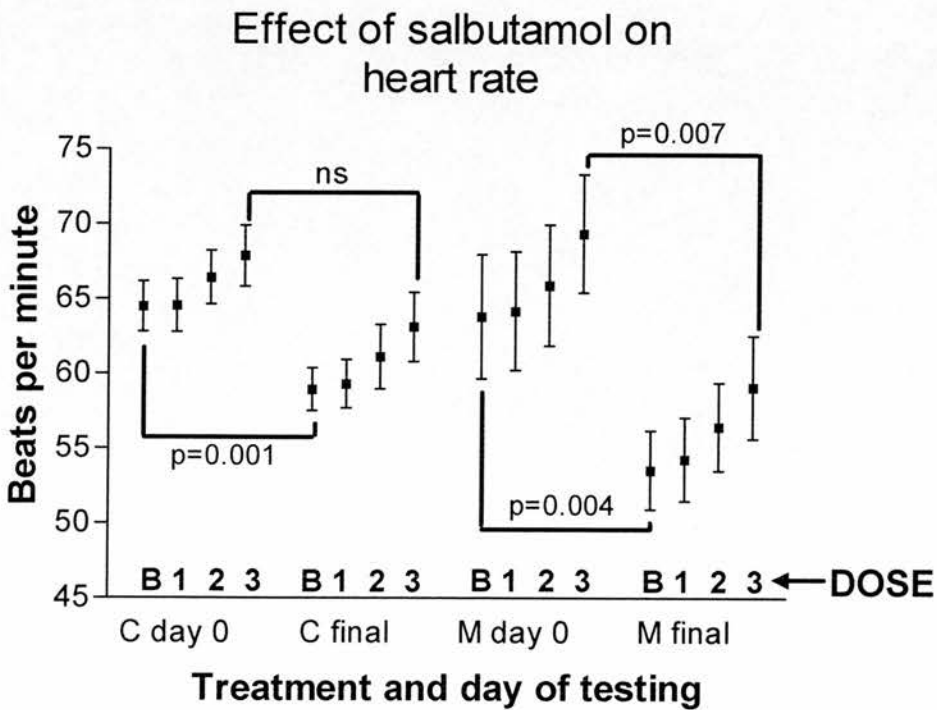


Figure 4-8 (see key above)



Effect of salbutamol on systolic blood pressure

Treatment and day of testing	Dose	Systolic Blood Pressure (mm Hg)
C day 0	B	~112.5
	1	~110.0
	2	~113.5
	3	~111.5
C final	B	~112.5
	1	~115.5
	2	~115.0
	3	~115.5
M day 0	B	~117.5
	1	~121.0
	2	~120.5
	3	~117.0
M final	B	~119.5
	1	~120.0
	2	~118.5
	3	~118.5

ns indicates no significant difference between C day 0 and C final, and between M day 0 and M final.

[illegible]

Figure 4-11 (see key above)

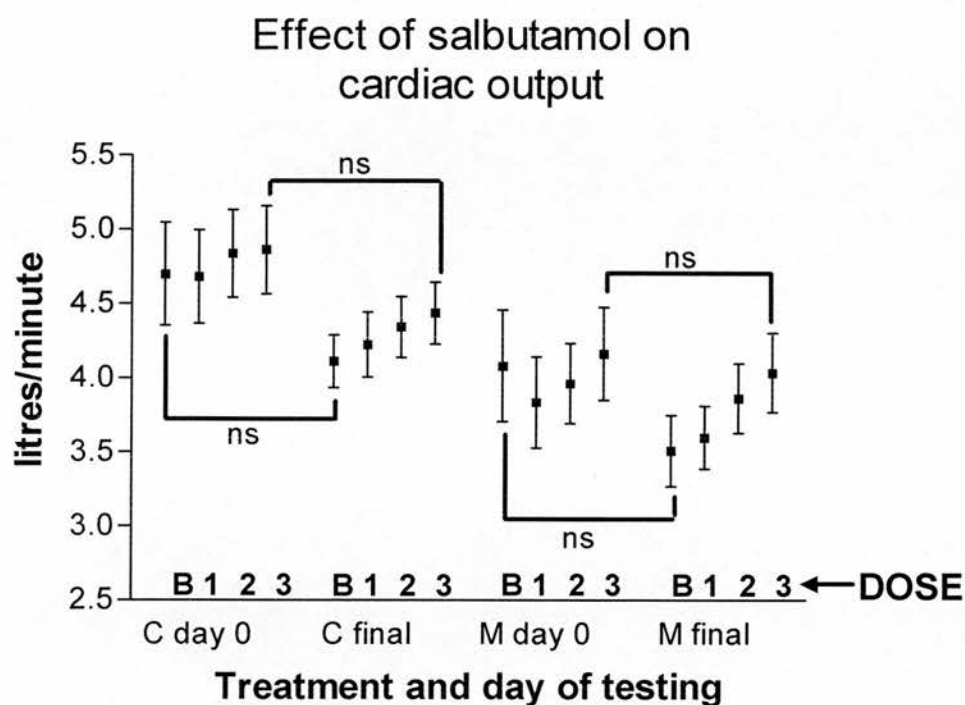
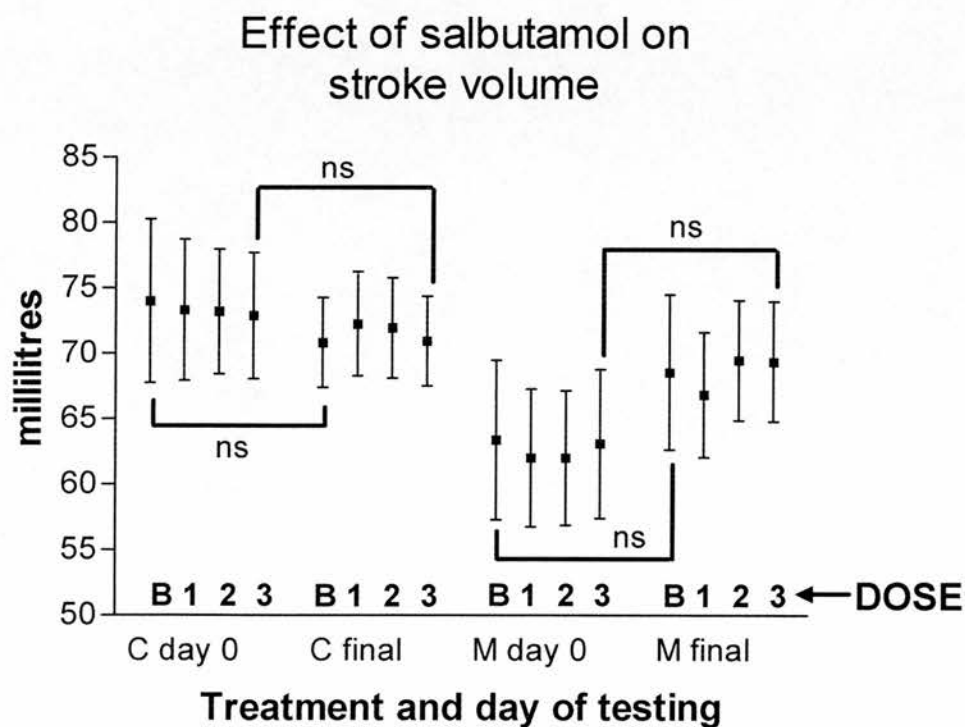


Figure 4-12 (see key above)



CHAPTER 5: PERIPHERAL ARTERIAL EFFECTS

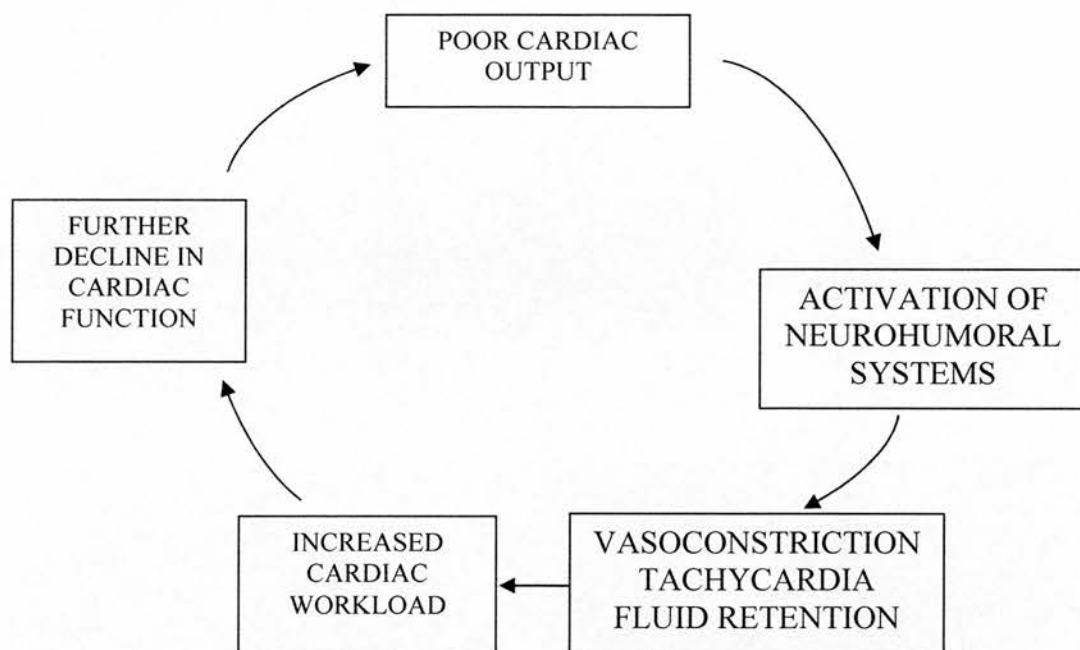
5.1. BACKGROUND

A cardinal haemodynamic feature of the syndrome of chronic heart failure is peripheral vasoconstriction and increased systemic vascular resistance.(Cowley, Stainer et al. 1986; Kubo, Rector et al. 1991; Katz, Biasucci et al. 1992) This is one of the mechanisms by which cardiac workload is increased in CHF and whereby the syndrome progresses (see Figure 5-1). Therefore, for a treatment to be of benefit, a desirable property of the agent would be an ability to reduce peripheral arterial tone. This was indeed the rationale behind the landmark “V-Heft” study,(Cohn, Archibald et al. 1986) which investigated the use of vasodilators for the treatment of CHF on the basis of the “haemodynamic hypothesis” of CHF. The subsequent introduction of ACE inhibitors, based in part on the results of the V-Heft II study,(Cohn, Johnson et al. 1991) meant that standard vasodilator therapy was thereafter largely obsolete. Nonetheless, there is still a place for the use of vasodilators in the management of some patients with CHF, especially those intolerant of agents acting on the renin-angiotensin-aldosterone axis, and possibly also in some patients with end-stage CHF.(Fonarow, Chelimskyfallick et al. 1992)

The ability to reduce arterial tone is an especially desirable property of agents acting on the adrenoreceptors, as in *untreated* CHF raised levels of circulating norepinephrine and increased sympathetic tone both contribute greatly to the increased systemic vascular resistance. Unfortunately, beta-blockers are known for their action to further increase peripheral vascular tone: by blocking the β_1 and β_2 adrenoreceptors (activation of which mediate vasodilatation in the periphery), the α -agonism of any endogenous adrenergic compound is unopposed resulting in further

increased vascular resistance. Quantifiable differences would be expected to exist between metoprolol and carvedilol in terms of their peripheral arterial β_1 and β_2 specificities.

Figure 5-1 : the vicious cycle of neurohumoral activation



Of great importance in interpretation of the peripheral arterial effects of drugs acting via these adrenoreceptors is the recognition that the peripheral adrenoreceptors have a very different population balance than cardiac adrenoreceptors. Whilst cardiac effects of adrenergic agonists are predominantly β_1 induced, the peripheral effects are mainly β_2 .

Carvedilol might be thought to have an advantage here, as its α_1 -adrenoreceptor blocking action may balance the β adrenoreceptor effect. In practice, it seems that the action of conventional beta-blockers to increase vascular tone does not cause a quantifiable effect in terms of worsening cardiac prognosis; the large trials of the more specific beta-blockers have achieved great success despite this theoretical increase in peripheral vascular tone. The most important clinical problem brought about by the beta-blocker effect is probably worsening symptoms of claudication in those with peripheral vascular disease.

A peripheral action to vasodilate via α receptors has long been thought to be worth pursuing. However, results with specific α receptor antagonists have been thoroughly disappointing, with the development of tachyphylaxis with prazosin in CHF (Packer, Meller et al. 1979) and with an increased incidence of clinical CHF being seen in the doxazosin arm of the ALLHAT trial. (ALLHAT Collaborative Research Group 2000) The use of α receptor antagonists to attempt to vasodilate in CHF has been abandoned.

The concept that a *short term* benefit can result from α receptor antagonism has evolved to try to explain the success of carvedilol; the theory is that short term vasodilatation reduces cardiac preload and afterload, so making the introduction of carvedilol (with its beta-blocking negative inotropism) more likely to be successful. Thus a component of this study was conceived to investigate the peripheral arterial α receptor antagonism of the two drugs under investigation.

5.2. OBJECTIVES

The objective of this part of the study was to investigate the actions of our investigational drugs on the peripheral arteries. By infusing relatively specific agonists for the three main adrenoreceptor types under study (α_1 , β_1 and β_2 receptors) we hoped to dissect out the various components of anti-adrenergic action of carvedilol and metoprolol on arterial tone. By using acetylcholine infusions and by inducing post-ischaemic hyperaemia, we also wished to determine if there were inherent differences between carvedilol and metoprolol on endothelial function.

5.3. PRINCIPLES OF VENOUS OCCLUSION PLETHYSMOGRAPHY

The technique of venous occlusion plethysmography (VOP) has been widely used to study forearm blood flow, as a model for general peripheral blood flow.(Whitney 1953; Greenfield, Whitney et al. 1963)Using this technique, one is able to study clinical pharmacology of vasoactive drugs in vivo rather than in vitro.(Benjamin, Calver et al. 1995) Of particular value in our study was the ability to assess the clinical pharmacology of patients suffering from CHF, rather than using a model of CHF.

The principle of the technique is that the rate of swelling of the forearm during occlusion of venous return is used to assess arterial *inflow* to the forearm. Provided perfusion pressure does not alter, changes in flow represent changes in smooth muscle tone in the small arteries and arterioles. Use of the brachial artery to infuse drugs permits investigation into the action of these agents on vascular tone, and in

this study the antagonist effect of a systemically administered drug (carvedilol or metoprolol) can be studied by administration of various specific agonists at the adrenoreceptor subtypes. The technique is most powerful, as in this study, when used to construct dose-response curves to different drugs within the same study.

VOP has been used to study forearm blood flow for more than 80 years, but modern technical developments have made the technique much less cumbersome. In particular, the use of mercury in silastic strain gauges was a great advance. A hollow loop of elastic filled with liquid mercury is used, the electrical conductance of which depends on the degree of distension. A voltage plethysmograph measures the minute changes in resistance. Due to safety concerns over the use of mercury, a liquid alloy of indium and gallium is now most commonly used. Analogue to digital signal converters (which permit almost immediate analysis of data on personal computers) accelerate analysis of recordings.

Total forearm blood flow is measured, which is comprised of skeletal muscle blood flow (50-70% of total) and skin blood flow. In this way, an approximation of total body blood flow is made. Strain-gauge plethysmography measures *total* flow in the forearm from wrist cuff to collecting cuff, and is not a measure of flow in the cross section of the arm immediately underneath it. The hands are excluded from assessment by tight wrist cuffs, as the blood flow in the hands is mainly cutaneous and its inclusion would detract from the accuracy of the procedure. Flow can be expressed per unit volume of forearm. The technique is well validated and is often used as the standard against which other methods are judged. (Benjamin, Calver et al. 1995)

The relationship between rate of increase in forearm volume and arterial flow only holds true if the forearm veins are not fully distended. Consequently, venous occlusion is performed for only approximately ten seconds at a time. When the veins are full, pressure will rise and blood will escape under the congesting cuff, so forearm volume no longer increases in proportion to arterial flow. If pressure in the forearm veins reaches 40 mm Hg, this begins to restrict arterial inflow.

The forearm is placed above the level of the right atrium so as to allow unimpeded venous return. Inflation of the upper arm venous occlusion cuff for 10 seconds in every 15 seconds causes a linear increase in forearm volume. After each inflation period, a 5 second deflation period allows emptying of the forearm veins. If flow rates become very high, it may sometimes be necessary to decrease the duration of the inflation period and increase the duration of the deflation period to avoid an excessive rise in venous pressure.

Vasoactive drugs alter the tone of the circular smooth muscle layer of the vessel wall, and only indirectly affect flow or resistance to flow. As it is not possible to directly assess tone of the vascular smooth muscle, the response to vasoactive substances is assessed indirectly by measuring changes in flow or derived changes in resistance. The extent to which altered contractile state of the vascular smooth muscle is translated into a change in flow depends on various factors: thickness of the muscle layer, vessel geometry, and initial conditions of resistance and flow.

Flow is a reasonable estimate of the smooth muscle tone provided arterial perfusion pressure does not change significantly in the course of the experiment. Due to autoregulation of circular smooth muscle tone (there is evoked contraction in

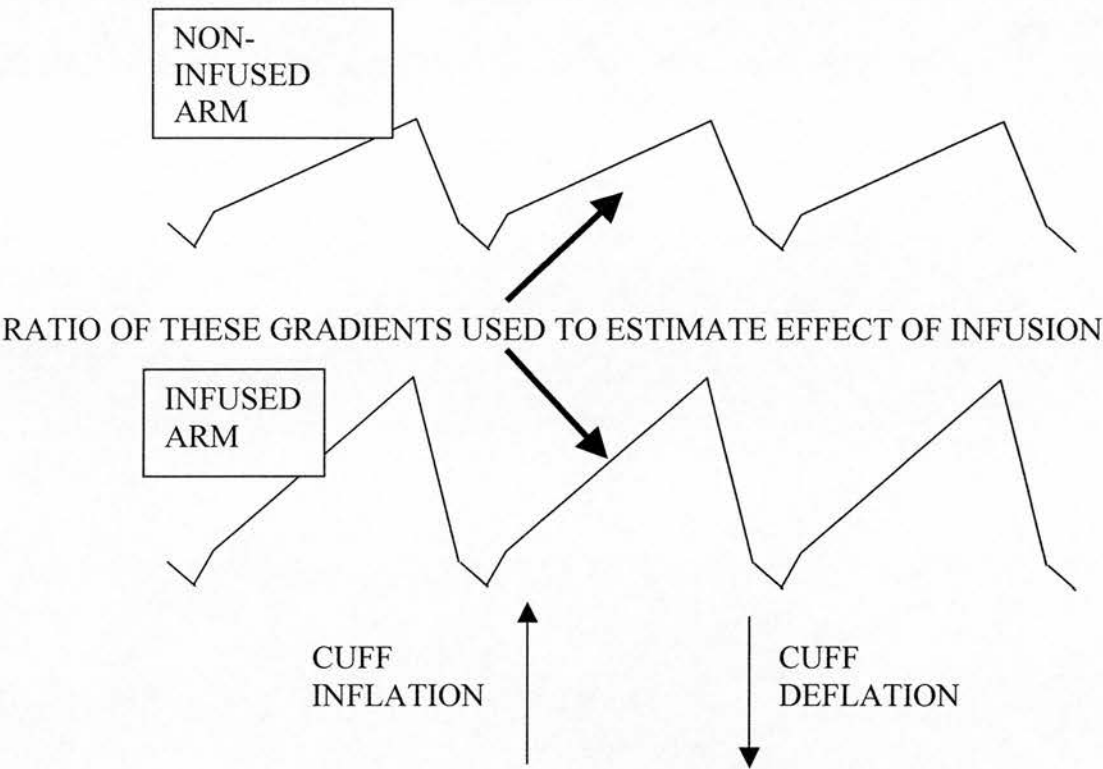
response to increased pressure), significant changes in arterial pressure make the results unreliable.

Use of simultaneous flow measurement in both the infused and the non-infused arm provides an internal control to the arrangement. The ratio of flow in the two arms approaches unity and stays constant even if blood flow alters markedly in response to changes in systemic arterial pressure or sympathetic arousal. Thus, the only difference in readings between the two arms will be as a consequence of the intervention being made on the infused arm. Any change in ratio of the blood flow between the two arms is a direct reflection of the change in local vascular tone in the test arm. In this way, if results are expressed in terms of the ratio of blood flow in the two arms, there is minimal variation and consistent and reproducible results are obtained. In this thesis, a comparison of the two arms is used rather than expression of the absolute values of forearm blood flow.

Recordings produced are of an upward slope during venous occlusion, the gradient of which is proportional to blood flow (see Figure 5-2). As mentioned above, the ratio of gradient of the slope from the infused (non-dominant) arm to that in the non-infused arm was equivalent to the ratio of blood flow between the two arms. The effect of drug infusion was expressed as percentage increase or decrease in blood flow, and was calculated by dividing the ratio of blood flow between the two arms at baseline. Due to the use of the non-infused arm as a control, extraneous influences (e.g. variations caused by temperature change, noise) were cancelled out and the only difference between the two arms was the difference between drug infusion and no

drug infusion. The technique has been shown to be highly reproducible with intra-subject variability of 19% between studies.

Figure 5-2: Schematic representation of plethysmographic recordings - effect of a vasodilator infusion



Drugs administered systemically have central effects, induce hormonal responses, change sympathetic output and alter blood pressure making changes in forearm blood flow difficult to interpret. Drug infusion through a 27 standard wire gauge needle placed in the brachial artery permits study of the direct vascular effects without affecting systemic arterial pressure. Doses used are often 100-1000 times lower than

systemically effective doses. Intermittent arterial pressure measurements were taken using a standard sphygmomanometer to demonstrate lack of systemic effects.

A cumulative dose-response curve can be produced to demonstrate the action of vasoactive substances. A linear relationship exists between the response and the log of the dose infused over a wide dose range. It is possible to construct dose-response curves for constrictors and for dilators with short half-lives. All the substances used in this study: phenylephrine, (Imaizumi, Harada et al. 1992; Sakai, Imaizumi et al. 1993; Indolfi, Maione et al. 1994) dobutamine, (Dawes, Chowienczyk et al. 1997) salbutamol (Dawes, Chowienczyk et al. 1997) and acetylcholine (Makimattila, Mantysaari et al. 1997; Tamai, Matsuoka et al. 1997)) have been shown to be appropriate for study in this manner. The area under the curve method can then be used for statistical comparison of dose response curves (see section 3.2).

Absolute values of forearm blood flow within an individual have a circadian rhythm, and changes can occur with a sudden change in sympathetic tone. These changes can affect the absolute response to drugs but will not alter the ratio of flow between the infused and the non-infused arm. Some variation of the response to drugs which are unstable in the blood e.g. acetylcholine, can occur even in the same subject on different days. It is generally recommended that results be expressed as a ratio of flow in the two arms.

5.4. RESULTS

It should be noted that all of the results here compare the effects of the various infusions on patients who have, at baseline on day 0, already received the first dose of study medication (carvedilol or metoprolol). As a result there is no true baseline value. This was an unavoidable consequence of the study design (see section 2.3). In addition to presenting area under the curve data, figures are also provided of data showing percentage change from baseline data (Figures 5-3 to Figure 5-6).

5.4.1. AREA UNDER THE CURVE ANALYSIS

5.4.1.1 EFFECT OF DOBUTAMINE

There is a significant effect of chronic treatment with carvedilol on the dose response of forearm blood flow to infusion of dobutamine (mean FBF reduced from 122 units to 37.6 units, $p=0.007$, 95% confidence limits for difference 29.7 – 139.4 units reduction) (see Table 5-1 and Figure 5-7). Metoprolol has no significant effect on the FBF response to dobutamine.

5.4.1.2 EFFECT OF SALBUTAMOL

There is also a highly significant effect of carvedilol on the dose response of forearm blood flow to infusion of salbutamol (mean FBF reduced from 1772 units to 566 units, $p=0.03$, 95% confidence limits for difference 150 – 2262 units reduction, see

Figure 5-8 and Table 5-2. Metoprolol has no significant effect on the FBF response to salbutamol.

5.4.1.3 EFFECT OF PHENYLEPHRINE

Again, after AUC analysis, neither chronic treatment with carvedilol nor metoprolol produce any significant change in the FBF response to intra-brachial phenylephrine infusion (see Table 5-3 and Figure 5-9).

5.4.1.4 EFFECT OF ACETYLCHOLINE

Chronic treatment with carvedilol nor metoprolol produces no significant change in the FBF response to intra-brachial acetylcholine infusion as assessed by AUC analysis (see Table 5-4 and Figure 5-10). There appears to be a *slight* trend for acetylcholine's vasodilatation to be inhibited by carvedilol.

5.4.1.5 EFFECT OF BETA-BLOCKERS ON ISCHAEMIC VASODILATATION

After 20 weeks of treatment both agents cause a significant reduction in the AUC ($p=0.012$ for carvedilol, $p=0.025$ for metoprolol). This indicates that forearm blood flow does not increase so much, and returns to normal more rapidly, after ischaemia when the subject is treated with either beta-blocker (see Table 5-5 and Figure 5-11).

5.5. DISCUSSION

This is the first time that the peripheral vascular effects of these two agents have been investigated in this comprehensive manner. Venous occlusion plethysmography has been widely used to study forearm blood flow as a model for general peripheral blood flow. Using this technique, one is able to study clinical pharmacology *in vivo* rather than *in vitro*. Of particular value in our study was the ability to assess the clinical pharmacology of patients suffering from CHF, rather than substituting a model for CHF. Use of the brachial artery to infuse drugs permitted investigation into the action of these agents on vascular tone, and in this study the antagonist effect of a systemically administered drug (carvedilol or metoprolol) was studied by administration of various agonists.

5.5.1. β ADRENORECEPTOR ACTIONS

Carvedilol acts in an anticipated manner to attenuate the vasodilator action of both dobutamine and salbutamol. This is much in keeping with the broad anti-adrenergic action expected of carvedilol, and is also be consistent with the findings in section 4.4. There it was found that carvedilol, whilst having less beta-blocking action than metoprolol in terms of heart rate reduction, nevertheless produced a definite blunting of the increase in stroke volume and cardiac output in response to stress.

Carvedilol appears to act against the general effect of dobutamine to a degree that exceeds metoprolol when one looks at cardiac output and forearm blood flow. The

action to inhibit the peripheral effect of dobutamine is corroborated in the few other studies to assess vascular tone (usually with systemic vascular resistance monitored invasively) during dobutamine infusion in carvedilol-treated patients. (Lowes, Simon et al. 2000; Lowes, Tsvetkova et al. 2001; Metra, Nodari et al. 2002)

Metoprolol has no significant effect on the FBF response to dobutamine, an effect that is at first glance unexpected. It would have been anticipated that the dobutamine action on forearm blood flow would have been attenuated after chronic metoprolol therapy, as metoprolol is much the more specific agent on the β_1 adrenoceptor. Other investigators, who found that the systemic vascular resistance response to dobutamine was essentially unaltered despite treatment with metoprolol, again corroborate this finding. (Metra, Nodari et al. 2002) It seems that carvedilol is indeed very much more effective than metoprolol in attenuating the peripheral response to dobutamine.

The marked difference seen between the effects of the two beta-blockers may be ascribed to their different mechanisms of action. Unlike metoprolol, carvedilol does not cause β_1 adrenergic receptor upregulation and it also blocks β_2 adrenergic receptors. (Bristow 2000) These properties, although potentially useful in the long-term, (Metra, Giubbini et al. 2000) may result in greater inhibition of the effects of dobutamine. In addition to its more comprehensive antiadrenergic action, carvedilol also exhibits “tight binding” to beta-adrenergic receptors, making it more difficult for an agonist to displace it. (Asano, Zisman et al. 2001)

Vasodilatation may have already occurred in the carvedilol-treated subjects to a degree that cannot be increased by the action of dobutamine, due to the action of carvedilol itself. Another explanation is the relative paucity of β_1 adrenoreceptors in the peripheral vasculature. This receptor subtype is well recognised to be important for positive inotropism and positive chronotropism, and much less important in terms of vasodilatation.

Metra also makes two suggestions:

1. Long-term metoprolol therapy may increase the β_1 adrenoreceptor density and improve signal transduction, so sensitizing the heart to β_1 activation. The sudden removal of metoprolol from the receptors by dobutamine then reveals the preserved or even accentuated dobutamine response.
2. Dobutamine has a reasonably high affinity for β_2 adrenoreceptors (Leier and Binkley 1998) which are left unoccupied by metoprolol. In addition, during chronic metoprolol administration, β_2 adrenoreceptors show improved coupling to intracellular transduction mechanisms through cross-regulation. (Hall, Kaumann et al. 1990) Thus chronic metoprolol therapy may actually accentuate the dobutamine effect.

Surprisingly, metoprolol attenuates the vasodilatory action of salbutamol more than it attenuates the vasodilatory effect of dobutamine. The effect is seen both during individual agonist level administrations and is also present during the AUC analysis, though fails to reach statistical significance. This effect is the converse of what

would be expected given the known beta-receptor actions of metoprolol. This finding is intriguing and is difficult to explain by simple adrenoreceptor specificities.

The most likely explanation is the distribution of the receptor subtypes with a relative paucity of peripheral β_1 receptors and of measurable β_1 receptor-induced peripheral action. Dobutamine may actually have very little peripheral β_1 effect, and the vasodilating effect it *does* may be manifested via its action on β_2 receptors rather than on β_1 receptors. Our observations are probably due to the lack of pure specificity of both metoprolol and dobutamine at β_1 receptors.

This peripheral vascular effect of metoprolol is certainly less surprising when its effects on heart rate are taken into account (see section 4.4). Within the confines of this study, there would appear to be a broader effect of metoprolol on beta-receptors than would be expected based only on its adrenoreceptor specificity.

5.5.2. α ADRENORECEPTOR ACTIONS

The comparison of carvedilol and metoprolol on phenylephrine-mediated vasoconstriction shows no statistically important differences between the two agents.

There are a number of possible explanations for this. It may be that the α_1 antagonism by carvedilol is very mild. It is also very possible that the main time of action of this effect has been missed by the study. At the start of the study, a very small dose of carvedilol is employed, which is likely to produce a negligible effect on α_1 receptors. It is also well recognised that tachyphylaxis develops to pure α_1 antagonism after

several weeks of therapy with specific α_1 antagonists in CHF (prazosin), so it is likely that any α_1 action of carvedilol has disappeared by the time of the repeat study at 20 weeks.

Other investigators have found that in normal individuals, acute administration of both carvedilol and metoprolol significantly attenuated the increased heart rate response to adrenergic stimuli when compared with effects of placebo.(Hryniewicz, Androne et al. 2003) Vascular resistance in the contralateral forearm during sustained isometric handgrip exercise is regulated by alpha-adrenergic-mediated vasoconstriction and concomitant neurogenic vasodilation.(Eklund and Kaijser 1976; Sanders, Mark et al. 1989) The cold pressor test induces sympathetic activation by a reflex neural pathway that augments α -adrenergic-mediated vasoconstriction in skeletal muscle circulations.(Greene, Boltax et al. 1965) In patients with heart failure, forearm blood flow response to isometric handgrip and cold pressor test did not change from baseline values in either treatment group and did not differ between treatment groups before or after 6 months of therapy. These data indicate that the α -adrenergic receptor blockade effects of carvedilol do not appear to play an important role in chronic therapy in subjects with heart failure. Indeed, other investigators have also deduced a decline in the alpha-blocking effect of carvedilol after chronic therapy. (Metra, Nardi et al. 1994; Kubo, Azevedo et al. 2001)

5.5.3. ENDOTHELIUM-DEPENDENT ACTIONS

5.5.3.1 ACTIONS ON ACETYLCHOLINE-MEDIATED VASODILATATION

Metoprolol has no effect on acetylcholine-mediated vasodilatation. This finding is as anticipated, as one would not expect an action of this agent on the cholinergic system, nor on the endothelium-dependent vasodilatation produced by acetylcholine.

There is a small but non-significant effect for long-term treatment with carvedilol to reduce acetylcholine-mediated vasodilatation. This effect of carvedilol is contrary to expectations: as an antioxidant, carvedilol would be expected to enhance nitric oxide-mediated vasodilatation, as the drug prevents the action of superoxide which normally neutralises nitric oxide. A number of publications have shown this effect. (Lopez, Christopher et al. 1995; Giugliano, Marfella et al. 1998; Matsuda, Akita et al. 2000) Carvedilol may also block production of factors counteracting nitric oxide-mediated vasodilatation e.g. endothelin-1.(Ohlstein, Arleth et al. 1998)

Some data exists to suggest there may be a component of the action of β_2 agonists that is actually mediated via endothelium-dependent mechanisms. Observers have reported a possible action of sympathetic ligands to alter endothelium-dependent vasodilatation.(Graves and Poston 1993; Wang, Poon et al. 1993; Ito, Yamamoto et al. 1997)

5.5.3.2 ACTIONS ON POST-ISCHAEMIC VASODILATATION

In contrast to this finding, carvedilol and metoprolol both reduce the area under the curve produced by measuring FBF post-ischaemic vasodilatation. This would suggest impairment of endothelial function with beta-blockade, or at least interference of the beta-blockers with some mechanism upon which endothelial-dependent dilatation relies. The magnitude of change is similar with carvedilol and metoprolol.

There is apparently contradictory evidence in this area. In one study of patients with coronary artery disease, carvedilol improved endothelial-dependent dilatation. (Matsuda, Akita et al. 2000) However, other investigators have shown that various sympathetic ligands such as isoprenaline (via α_1), salbutamol and dobutamine all act via endothelium (nitric oxide)-dependent pathways to induce vasodilatation. (Graves and Poston 1993; Wang, Poon et al. 1993; Ito, Yamamoto et al. 1997) One might therefore expect that beta blockers would impair this ability to relax vessels via endothelium-dependent paths.

The fact that there appears to be a contradiction here is not so surprising, as the action of acetylcholine to vasodilate is of course mediated via nitric oxide (and probably via other endothelial-derived products). This mechanism is well elucidated, and there would be no obvious point at which a beta-blocker might interfere. Whilst the main stimulus for vasodilatation during acetylcholine infusion is always the acetylcholine, the main stimulus after ischaemia is a generalised activation of endothelial factors. Some of these factors could conceivably be influenced by local adrenergic status.

5.6. CONCLUSION

This study involved a small number of patients, so there was a large variance for some measures making it impossible to be certain that there is no difference between the groups in some measures. It is also possible some of the apparently statistically significant changes shown are spurious.

Metoprolol has a much more limited action on peripheral β_1 -receptor-mediated vasodilatation than is expected from its pharmacological properties. However, this finding is consistent with other observers. Carvedilol significantly inhibits peripheral vasodilatation in response to both dobutamine and salbutamol to a statistically significant degree. In having this action, carvedilol has a very much more significant generalised beta-blocking action on the periphery than does metoprolol. This would have been anticipated from the known adrenoreceptor specificities of the drug. The β_2 receptor is a far more important subtype than β_1 in the periphery, and is obviously much more influenced by carvedilol than by metoprolol.

If any significant vasodilating alpha-receptor-mediated action of carvedilol exists, this study was not able to detect this effect. It is conceivable that this is due to the timing of the study observations.

The action of carvedilol to vasodilate has been much vaunted as a means of better carvedilol tolerability during initiation, the theory being that vasodilatation reduces cardiac workload and so means that a patient having a beta-blocker initiated would be less likely to go into worsening heart failure. In this study, no important

vasodilating action of carvedilol was found possibly due in part to the limited time points at which observations were made. The action of carvedilol is rather to prevent adrenergic agonist-mediated vasodilatation at least after chronic therapy.

Table 5-1: areas under the curve for dobutamine infusions

	n	Mean	s.d.	% change	s.e.	95% CI for diff.	T-value	P-value
Carvedilol Day 0	10	122.3	75		24			
Carvedilol Final day difference	10	37.6	78.9		25			
	10	84.6	76.6	-69.3%	24.2	29.7, 139.4	3.49	0.007
Metoprolol Day 0	6	214.8	160		65.3			
Metoprolol Final day difference	6	195.1	89.4		36.5			
	6	19.7	100.5	-9.2%	41	-85.8, 125.1	0.48	0.652

Table 5-2: areas under the curve for salbutamol infusions

	n	Mean	s.d.	% change	s.e.	95% CI for diff.	T-value	P-value
Carvedilol Day 0	10	1772	1463		462			
Carvedilol Final day difference	10	566	553		175			
	10	1206	1476	-68.1%	467	150, 2262	2.58	0.030
Metoprolol Day 0	6	2138	991		404			
Metoprolol Final day difference	6	1257	848		346			
	6	881	945	-41.2%	386	-110, 1873	2.29	0.071

Table 5-3: areas under the curve for phenylephrine infusions

	n	Mean	s.d.	% change	s.e.	95% CI for diff.	T-value	P-value
Carvedilol Day 0	10	78.7	75.7		23.9			
Carvedilol Final day difference	10	40.4	26.7		8.4			
	10	38.4	74.9	-48.8	23.7	-15.2, 92.0	1.62	0.140
Metoprolol Day 0	6	50.1	43.1		17.6			
Metoprolol Final day difference	6	38.7	18.5		7.6			
	6	11.4	43.5	-22.8	17.8	-34.3, 57.0	0.64	0.550

Table 5-4: areas under the curve for acetylcholine infusions

	n	Mean	s.d.	% change	s.e.	95% CI for diff.	T-value	P-value
Carvedilol Day 0	10	-1.953	0.932		0.295			
Carvedilol Final day	10	-1.475	1.061		0.336			
Carvedilol difference	10	-0.478	1.106	24.5%	0.350	-1.269, 0.313	-1.37	0.205
Metoprolol Day 0	6	-1.825	1.536		0.627			
Metoprolol Final day	6	-1.097	1.050		0.429			
Metoprolol difference	6	-0.727	1.772	39.8%	0.723	-2.587, 1.132	-1.01	0.361

Table 5-5: Paired T tests for ischaemic vasodilatation (area under the curve analysis)

	n	Mean	s.d.	% change	s.e.	95% CI for diff.	T-value	P-value
Carvedilol Day 0	8	9922	3088		1092			
Carvedilol Final day	8	6332	2535		896			
Carvedilol difference	8	3590	3017	-36.2%	1067	1066, 6114	3.37	0.012
Metoprolol Day 0	5	13097	2583		1155			
Metoprolol Final day	5	8654	2065		923			
Metoprolol difference	5	4443	2843	-33.9%	1272	913, 7973	3.49	0.025

Figure 5-3

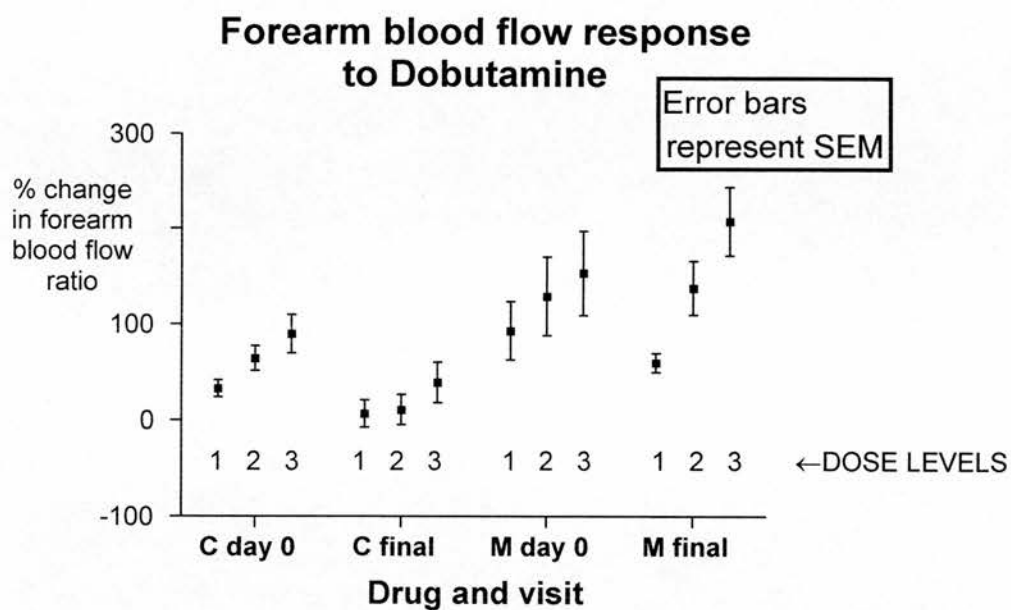


Figure 5-4

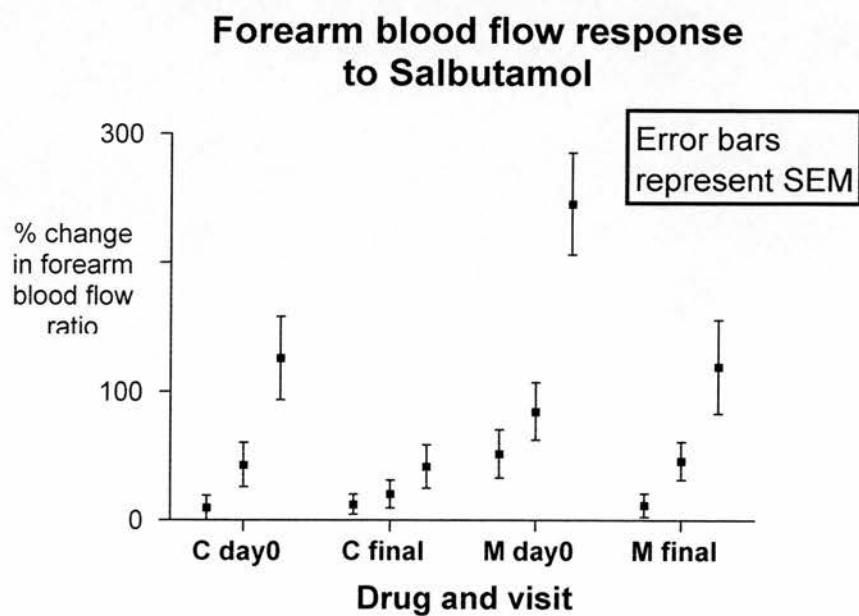


Figure 5-5

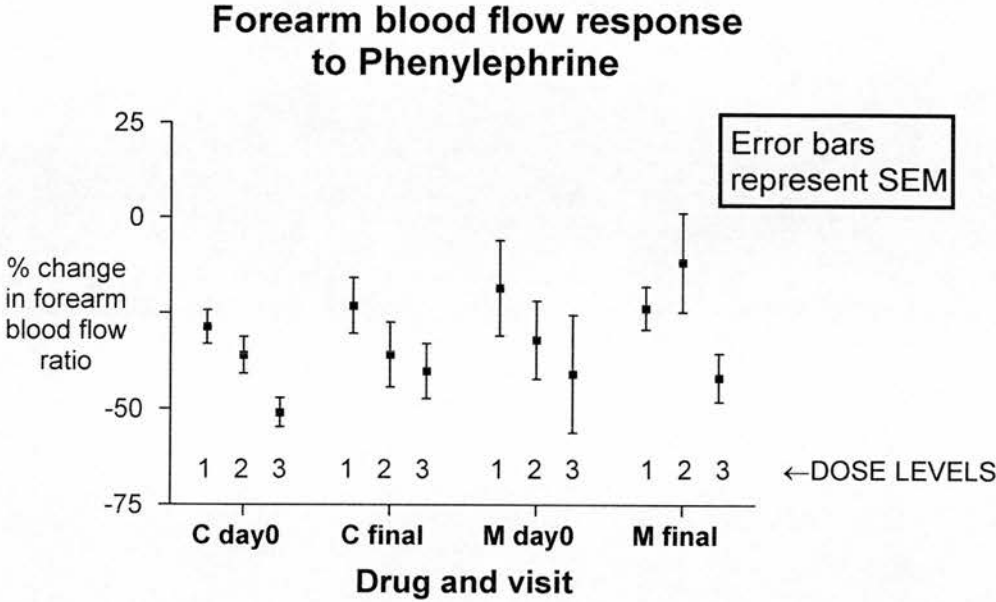


Figure 5-6

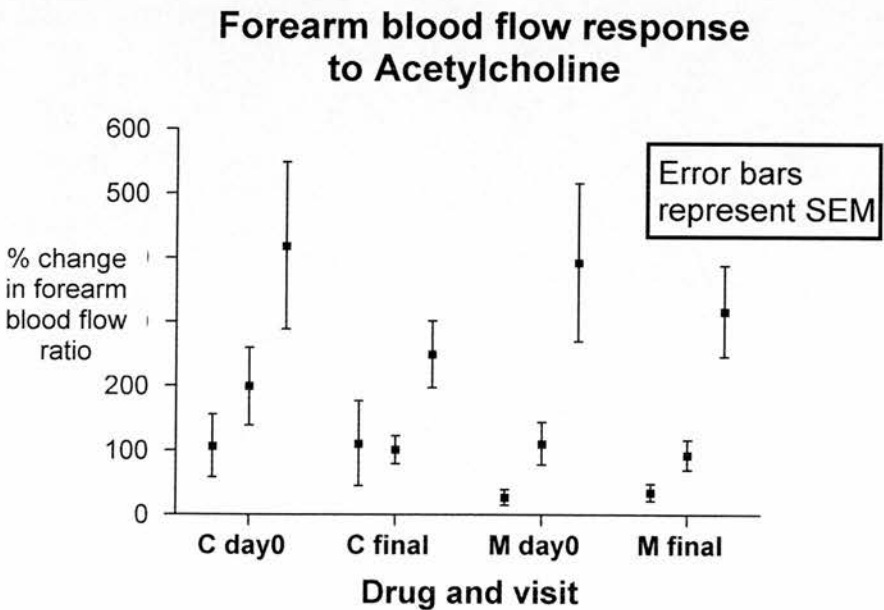


Figure 5-7: comparison of the areas under the curve during dobutamine infusion

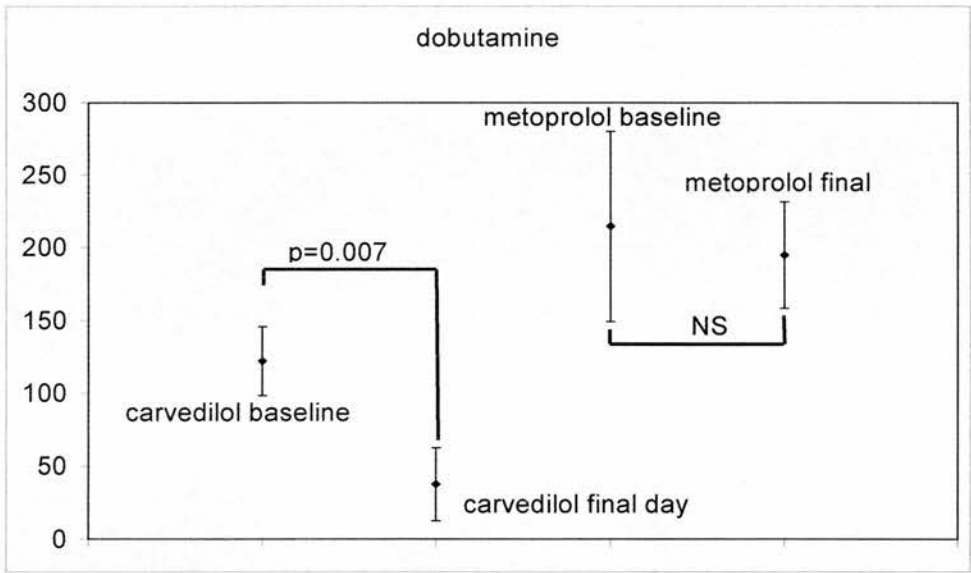


Figure 5-8: comparison of the areas under the curve during salbutamol infusion

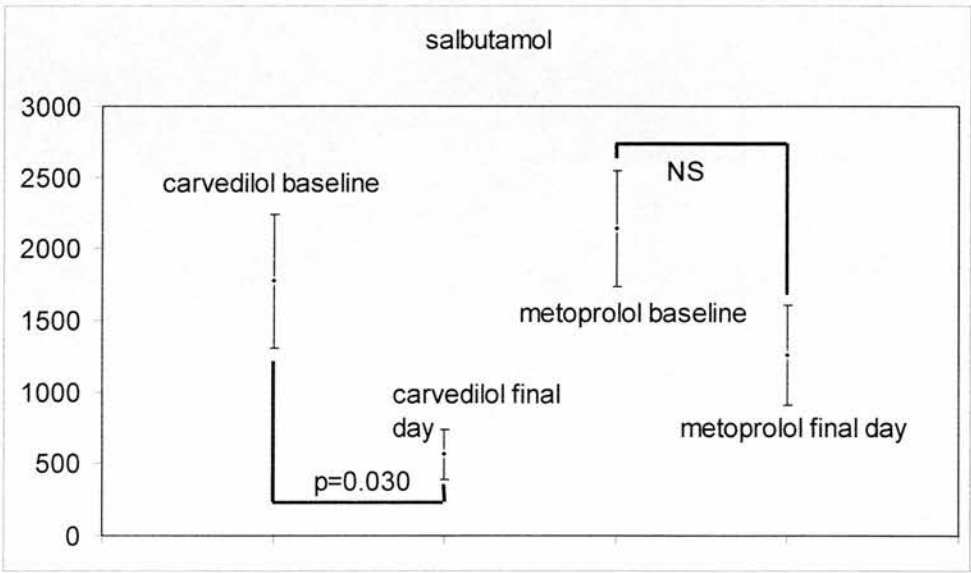


Figure 5-9: comparison of areas under the curve for phenylephrine infusion

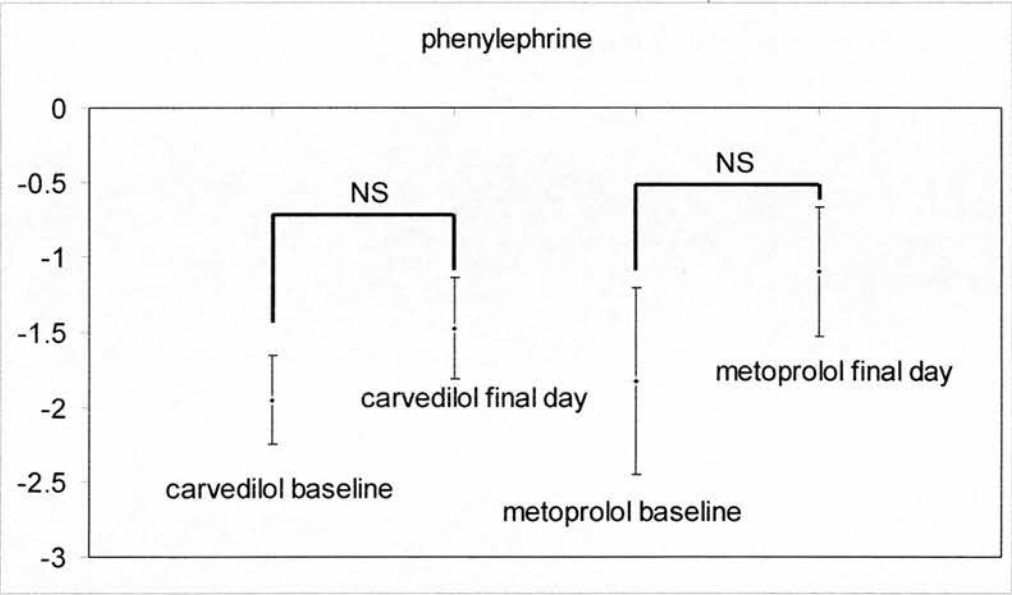


Figure 5-10: comparison of areas under the curve for acetylcholine

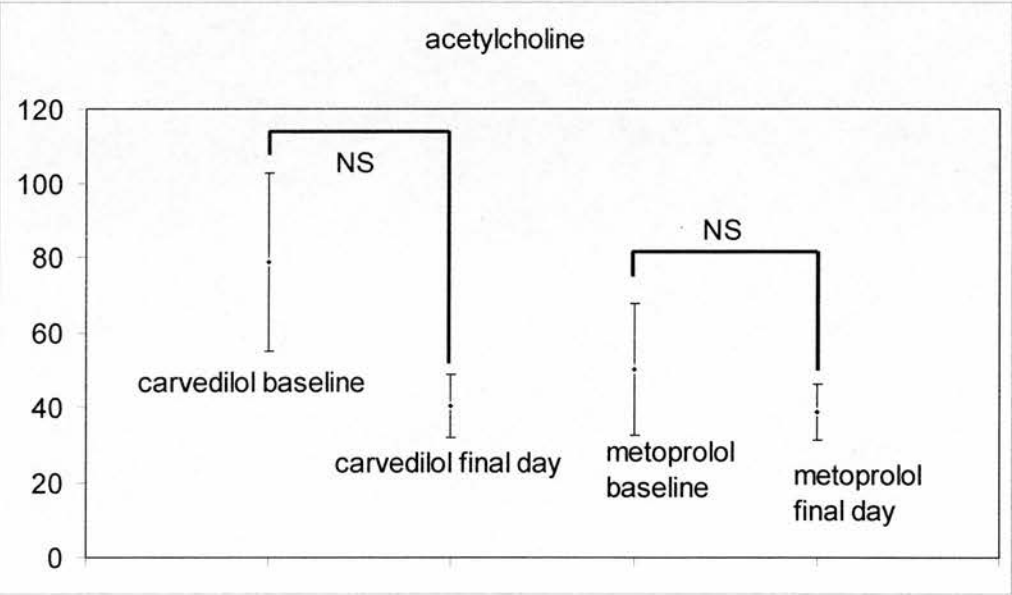
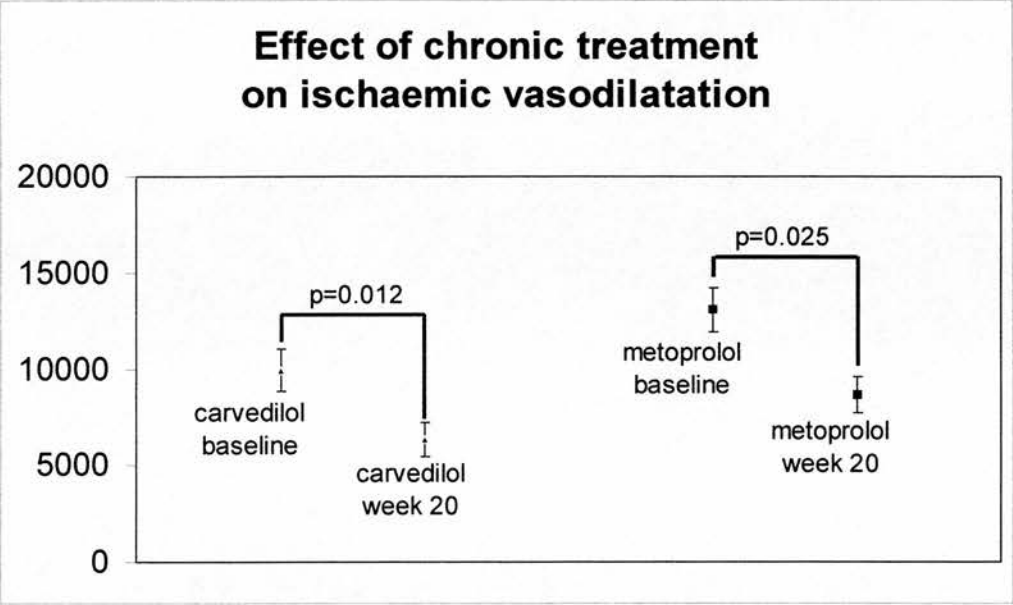


Figure 5-11: comparison of response to ischaemic vasodilatation



CHAPTER 6: NEUROHUMORAL MEASURES

6.1. OBJECTIVE

We aimed to examine the neurohumoral alterations in CHF caused by treatment with carvedilol and metoprolol.

Neurohumoral responses to reduced cardiac output participate in the pathogenesis of CHF (see section 1.4.2). Both basal and exercise-induced concentrations of endogenous neurohumoral substances are elevated in patients with CHF compared with normal controls.(Kjaer, Appel et al. 2004) Plasma concentrations of several neurohumoral factors have also been extensively studied with regard to their prognostic value in CHF.(Cohn, Levine et al. 1984; Francis, Cohn et al. 1993; Parmley 1995; Frey, Pacher et al. 2000; McDonagh, Cunningham et al. 2001)

It is of obvious interest to determine whether there are differential actions by carvedilol and metoprolol on these parameters. Assuming the neurohormonal hypothesis of chronic heart failure is at least partly true, the influence of proven beneficial CHF therapies on neurohumoral measures should be predictable.

There are in fact very few placebo-controlled studies of the neurohumoral actions of beta-blockers in CHF.(Holmer, Hense et al. 1998; Jansson, Dahlstrom et al. 1999) We know of only two other comparisons of the neurohumoral effects of carvedilol compared to metoprolol, (Hirooka, Yasumura et al. 2001; Fung, Yu et al. 2003) and in neither of these studies was therapy blinded in any way. The first study examines only the effects of the twoi drugs on natriuretic peptide concentrations. Fung et al. performed a much more comprehensive study, investigating a much broader panel of measures.(Fung, Yu et al. 2003) The true effect of beta-blockers on neurohumoral measures remains unclear.

6.2. METHODS

As explained in chapter 2, blood sampling was performed at three points during the study:

- Just prior to initiation of first dose of beta-blocker therapy
- After 10 weeks (at the end of the titration period)
- After 20 weeks (at the end of the maintenance period)

Subjects attended the study day having abstained from alcohol, caffeine and tobacco for 24 hours, from food for 6 hours, and having emptied their bladder. Under local anaesthesia (to minimise neurohumoral activation) a 16-gauge cannula was sited in the antecubital vein of the dominant arm to enable blood sampling. The subjects were then allowed to lie supine on a couch for 20 minutes for equilibration to occur: this is important to prevent postural variation in neurohumoral activation, which could theoretically invalidate the results of samples taken. Blood samples were then taken into chilled tubes containing the appropriate preservatives for neurohumoral tests.

6.2.1. NEUROHUMORAL ASSAYS

Dianne Hillyard performed these assays in the laboratory of Dr Ian Morton in the department of Medicine and Therapeutics at the Western Infirmary, Glasgow.

6.2.1.1 NATRIURETIC PEPTIDES

Brain natriuretic peptide was assayed without prior extraction of plasma by means of a direct, specific monoclonal antibody radioimmunoassay kit supplied by Shionogi & Co, Ltd (Settsu-shi, Osaka, Japan), as previously described.(Murdoch, Byrne et al. 1997) Plasma N-terminal atrial natriuretic peptide (ANP 1-30) was assayed by radioimmunoassay after extraction from acidified plasma with C18 reverse-phase columns (Sep-Pak, Waters Associates Ltd, Watford, UK) with the use of a modification of a previously described method. (Richards, Tonolo et al. 1987; McDonagh, Robb et al. 1998) A Peninsula Laboratories (Belmont, California, USA) antibody, RAS 9129 was used for identification of N-terminal atrial natriuretic peptide. The between-assay coefficients of variation were 15% for the N-terminal atrial natriuretic peptide assay and <10% for the brain natriuretic peptide assay, respectively.

6.2.1.2 PLASMA RENIN ACTIVITY

Plasma renin activity was measured by an in-house antibody trapping technique in the presence of added excess renin substrate.(Millar, Leckie et al. 1980) The coefficient of variation was 3.4%.

6.2.1.3 ANGIOTENSIN II

An in-house radioimmunoassay was used for plasma angiotensin II as previously described.(Morton and Webb 1985) Angiotensin II was preextracted from plasma before assay. The coefficient of variation was 10%.

6.2.1.4 ALDOSTERONE

Plasma aldosterone was measured with a solid-phase (coated tube) radioimmunoassay kit supplied by Diagnostic Products (UK) Ltd. The coefficient of variation was <8.3%.

6.2.1.5 NOREPINEPHRINE

Plasma norepinephrine (nmol/l) was extracted from plasma and assayed by high performance liquid chromatography and electrochemical detection as previously described.(Goldstein, Feuerstein et al. 1981; McAlpine, Morton et al. 1988) The within assay and between assay coefficients of variation are both < 10%.

6.2.1.6 EPINEPHRINE

Plasma epinephrine was extracted from plasma and assayed by high performance liquid chromatography and electrochemical detection as previously described.(Goldstein, Feuerstein et al. 1981; McAlpine, Morton et al. 1988) The within assay and between assay coefficients of variation are both < 10%.

6.2.1.7 ENDOTHELIN

Plasma endothelin concentration was measured by the method of Davenport et al. (Davenport, Ashby et al. 1990)as previously described.(McMurray, Ray et al. 1992) The lower limit of detection of the assay is 0.5pmol/l.

6.3. RESULTS

There are a number of significant differences in the neurohumoral effects of carvedilol and metoprolol during the course of this study.

6.3.1. BASELINE NEUROHUMORAL MEASURES

Baseline measurements of neurohumoral activity for patients completing the study are shown in Table 6-1.

Table 6-1: baseline neurohumoral levels

NEUROHUMORAL MEASURES	Metoprolol mean ± s.d.	Carvedilol mean ± s.d.	P value for difference
norepinephrine (nmol/L)	4.71±1.53	4.03±1.12	0.204
epinephrine (nmol/L)	0.30±0.39	0.15±0.13	0.213
renin (μU/ml)	299±341	391±632	0.619
angiotensin II (pg/ml)	8.8±10.3	7.5±7.2	0.697
aldosterone (ng/ml)	11.1±4.5	17.5±16.4	0.209
ANP (ng/ml)	8.3±8.7	6.6±4.9	0.555
BNP (pg/ml)	80±97	85±65	0.881
ET-1 (fmol/ml)	0.37±0.22	1.21±2.60	0.201

6.3.2. **ATRIAL NATRIURETIC PEPTIDE**

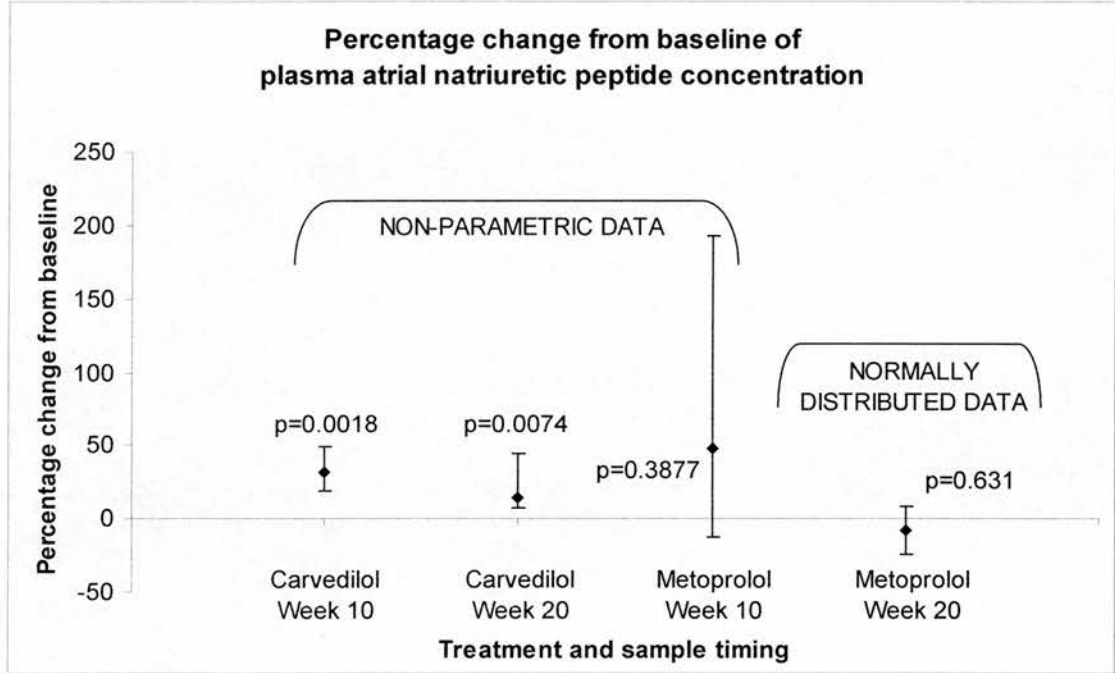
Carvedilol produces a statistically significant increase in plasma atrial natriuretic peptide concentration after both ten and twenty weeks of treatment. No clear effect is seen with metoprolol (see Table 6-2 and Figure 6-1).

Table 6-2 : Effect of treatment on plasma atrial natriuretic peptide concentration

Atrial natriuretic peptide						
Percentage change from baseline	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P- value
	Carvedilol Week 10	16	31.75%	18.36	49.37	0.0018
	Carvedilol Week 20	16	14.59%	6.52	44.42	0.0074
	Metoprolol Week 10	12	48.53%	-13.2	192.9	0.3877
	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	T-value	P- value
	Metoprolol Week 20	12	-8.1%	16.34%	0.49	0.631

Figure 6-1. Effect of treatment on plasma atrial natriuretic peptide concentration

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.



6.3.3. B-TYPE NATRIURETIC PEPTIDE

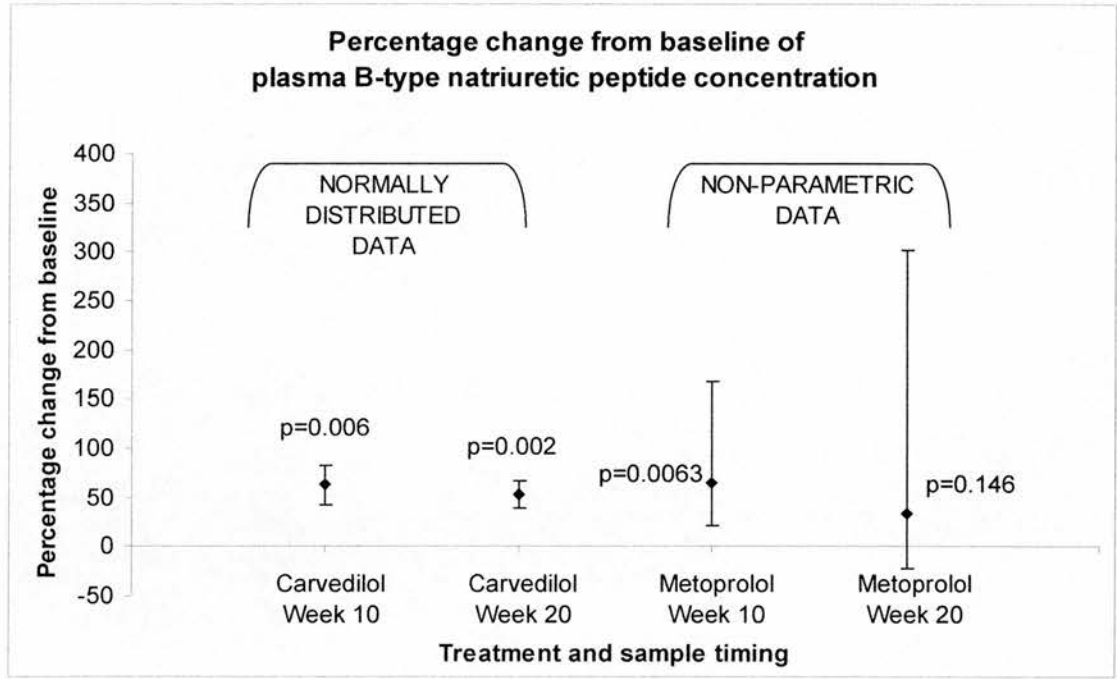
Both beta-blockers produce marked increases in plasma BNP concentration and the increases are of similar magnitude. The effects are seen at both 10 and twenty weeks though the metoprolol effect fails to reach statistical significance at 20 weeks (see Table 6-3 and Figure 6-2).

Table 6-3 : Effect of treatment on plasma B-type natriuretic peptide concentration

B-type natriuretic peptide						
Percentage change from baseline	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	T- value	P- value
	Carvedilol Week 10	17	62.9%	20.08%	-3.13	0.006
	Carvedilol Week 20	17	52.6%	13.91%	-3.78	0.002
	NON- PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P- value
	Metoprolol Week 10	12	64.27%	21.7	167.9	0.0063
	Metoprolol Week 20	12	34.49%	-22.0	302.7	0.1460

Figure 6-2. Effect of treatment on plasma B-type natriuretic peptide concentration

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.



6.3.4. RENIN

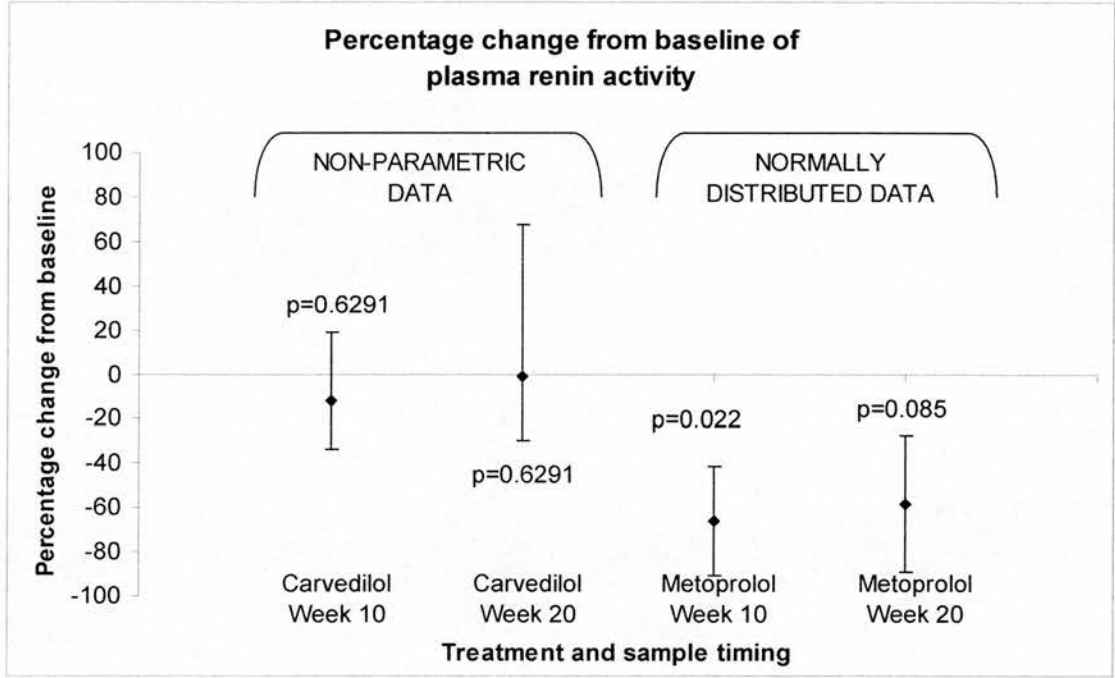
Carvedilol has no effect on plasma renin activity. Metoprolol markedly reduces plasma renin activity (see Table 6-4 and Figure 6-3).

Table 6-4: Effect of treatment on plasma renin activity

Plasma Renin activity						
Percentage change from baseline	NON-PARAMETRIC DATA (1-sample sign test)					
		n	Median	95% CI (lower)	95%CI (upper)	P- value
	Carvedilol Week 10	17	-12.16%	-33.30	19.24	0.6291
	Carvedilol Week 20	17	-1.25%	-30.08	67.58	0.6291
	NORMALLY DISTRIBUTED DATA (t-test)					
		n	Mean	s.e.	T- value	P- value
	Metoprolol Week 10	12	-65.8%	24.72%	2.66	0.022
	Metoprolol Week 20	12	-58.3%	30.79%	1.89	0.085

Figure 6-3. Effect of treatment on plasma renin activity

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.



6.3.5. ANGIOTENSIN II

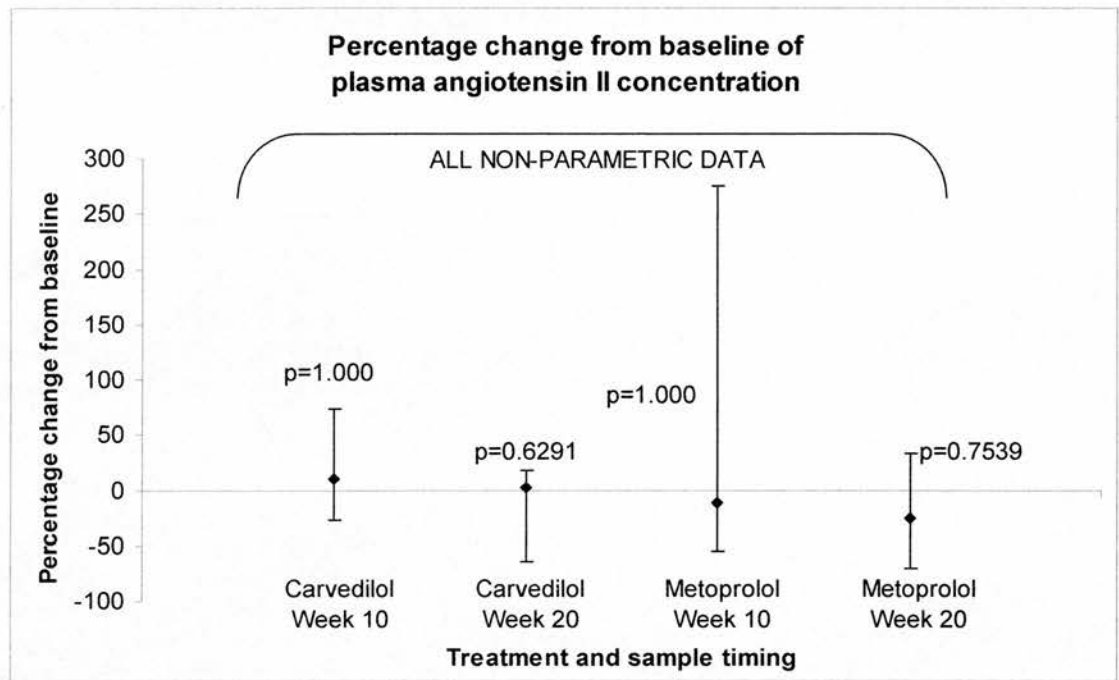
Carvedilol tends to increase plasma angiotensin II concentration, whilst metoprolol tends to decrease the concentration (see Table 6-5 and Figure 6-4). Neither effect reaches statistical significance.

Table 6-5: Effect of treatment on plasma angiotensin II concentration

Angiotensin II	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P-value
Percentage change from baseline	Carvedilol Week 10	17	10.20%	-27.59	73.93	1.000
	Carvedilol Week 20	17	2.04 %	-64.18	17.38	0.6291
	Metoprolol Week 10	12	-11.76%	-55.8	275.6	1.000
	Metoprolol Week 20	12	-25.29%	-69.82	33.86	0.7539

Figure 6-4. Effect of treatment on plasma angiotensin II concentration.

The points represent medians and bars represent 95% confidence intervals.



6.3.6. ALDOSTERONE

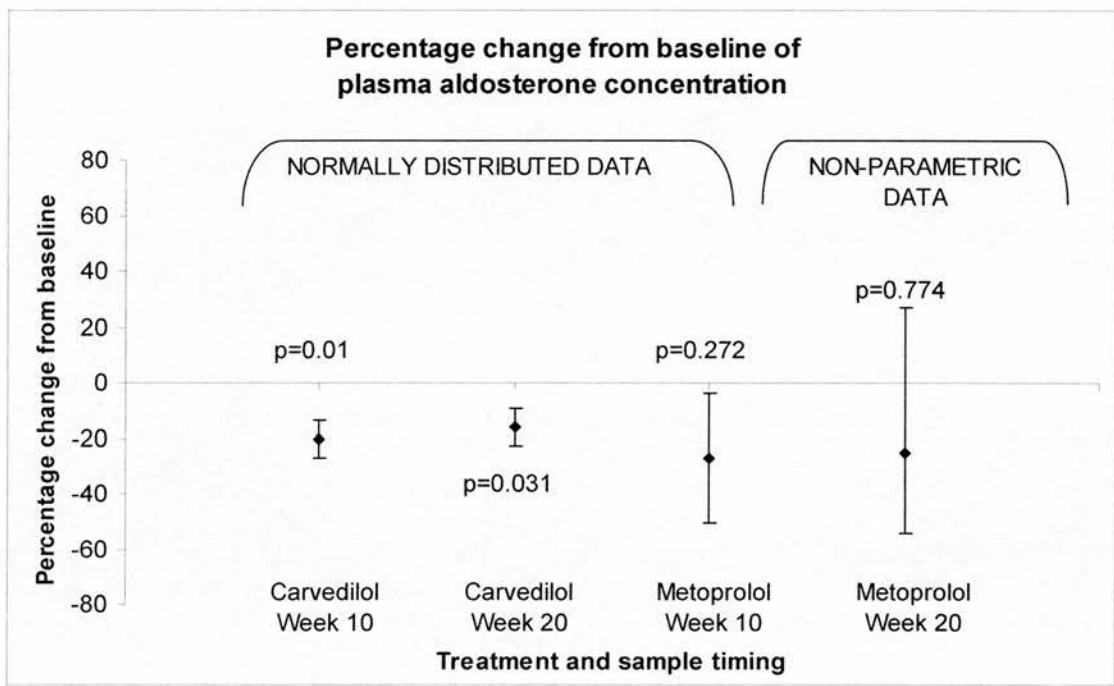
Both carvedilol and metoprolol produce a decrease in plasma aldosterone concentration, and this effect is of similar magnitude in both groups (see Table 6-6 and Figure 6-5). The effect is, however, only statistically significant in the carvedilol group at both 10 and 20 weeks.

Table 6-6 : Effect of treatment on plasma aldosterone concentration

Aldosterone						
Percentage change from baseline	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	T-value	P-value
	Carvedilol Week 10	17	-20.2%	6.87%	2.94	0.010
	Carvedilol Week 20	17	-16.0%	6.74%	2.37	0.031
	Metoprolol Week 10	12	-27.1%	23.48%	1.16	0.272
	NON- PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P-value
	Metoprolol Week 20	12	- 25.40%	-54.09	26.88	0.774

Figure 6-5. Effect of treatment on plasma aldosterone concentration.

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.



6.3.7. **NOREPINEPHRINE**

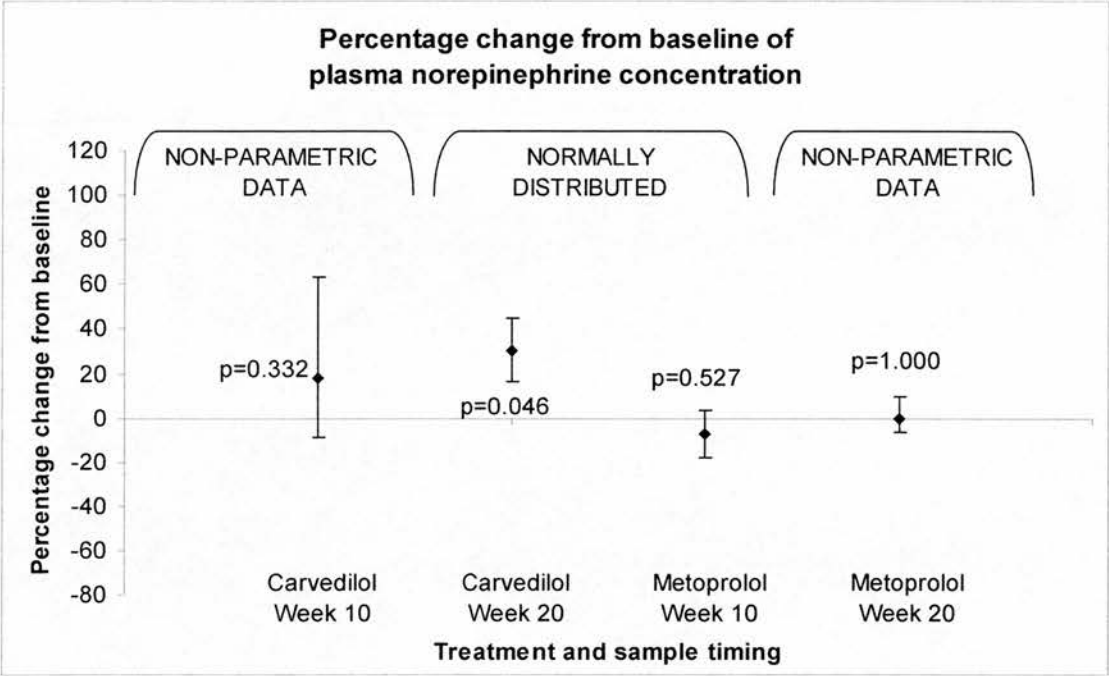
Carvedilol significantly increases plasma norepinephrine concentration at twenty weeks. No such effect is seen with metoprolol (see Table 6-7 and Figure 6-6).

Table 6-7: Effect of treatment on plasma norepinephrine concentration

Norepinephrine						
Percentage change from baseline	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P- value
	Carvedilol Week 10	17	18.18%	-8.73	63.26	0.332
	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	T- value	P- value
	Carvedilol Week 20	17	30.7%	14.21%	-2.16	0.046
	Metoprolol Week 10	12	-7.1%	10.83%	0.65	0.527
	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P- value
	Metoprolol Week 20	12	0%	-6.546	9.291	1.000

Figure 6-6. Effect of treatment on plasma norepinephrine concentration.

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.



6.3.8. EPINEPHRINE

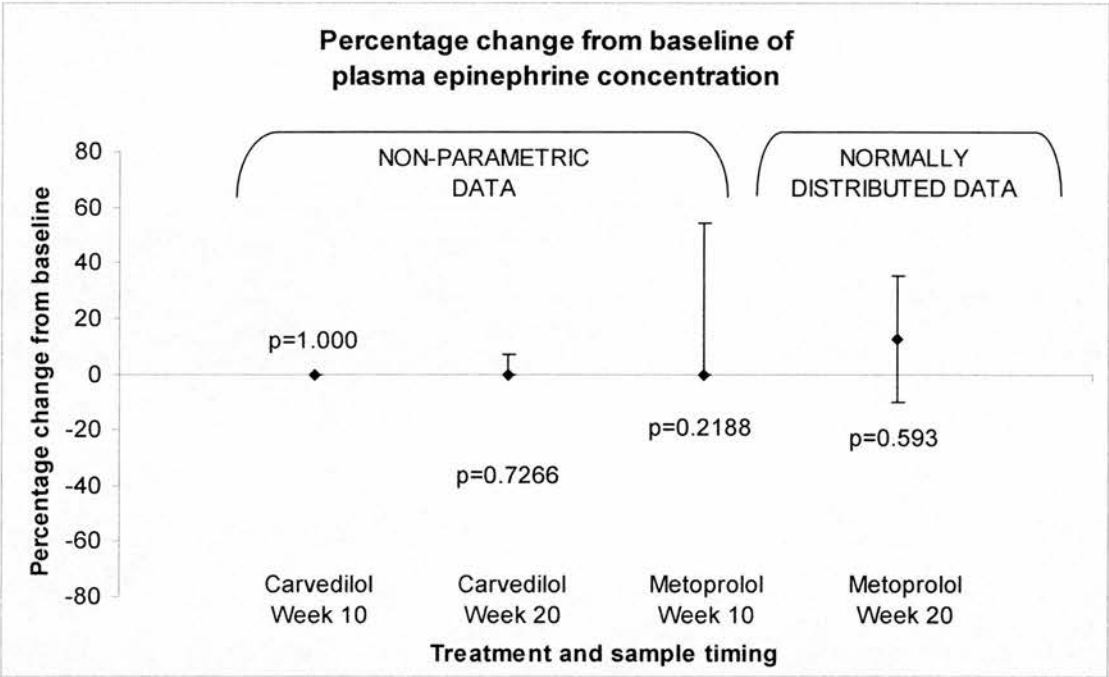
There was no statistically significant change in plasma epinephrine concentration over the course of the study in the individual groups. Due to the nature of the assay for plasma epinephrine concentration, the data appears skewed(see Table 6-8 and Figure 6-7).

Table 6-8 : Effect of treatment on plasma epinephrine concentration

Epinephrine						
Percentage change from baseline	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P-value
	Carvedilol Week 10*	16	0%	0	0	1.0000
	Carvedilol Week 20*	16	0%	0	6.971	0.7266
	Metoprolol Week 10	12	0%	-54.29	0	0.2188
	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	T-value	P-value
	Metoprolol Week 20*	11	12.5%	22.7	0.55	0.593
* single outlier excluded during analysis						

Figure 6-7. Effect of treatment on plasma epinephrine concentration

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.



6.3.9. **ENDOTHELIN**

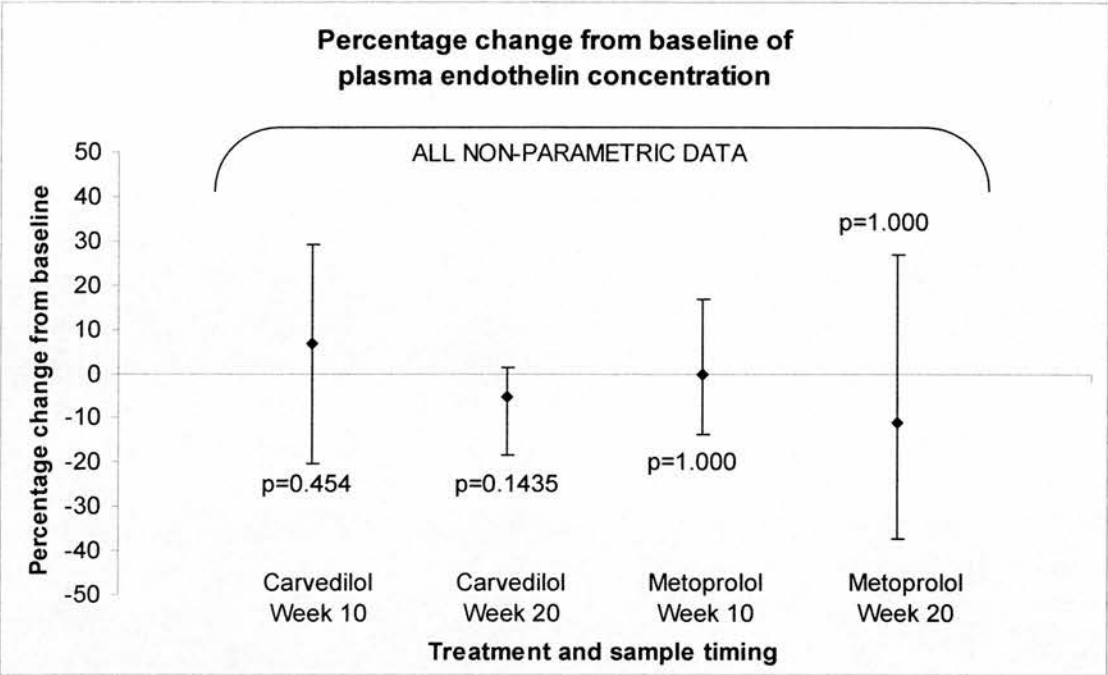
Neither agent has a significant influence on plasma endothelin concentration (see Table 6-9 ad Figure 6-8).

Table 6-9: Effect of treatment on plasma endothelin concentration

Endothelin	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P-value
Percentage change from baseline	Carvedilol Week 10	17	6.67%	-20.08	29.16	0.4545
	Carvedilol Week 20	17	-5.26%	-18.47	1.47	0.1435
	Metoprolol Week 10	12	0%	-13.76	16.76	1.0000
	Metoprolol Week 20	12	-11.19%	-37.40	26.89	1.0000

Figure 6-8. Effect of treatment on plasma endothelin concentration.

The points represent medians and bars represent 95% confidence intervals.



6.4. DISCUSSION

As with the other analyses, this study involved a small number of patients, so there was a large variance for some measures making it impossible to be certain that there is no difference between the groups in some measures. It is also possible some of the apparently statistically significant changes shown are spurious.

Interpretation of the effects of drugs on neurohumoral measures is difficult. Untreated CHF patients are likely to have high levels of e.g. norepinephrine or BNP. However, whether the action of a treatment is beneficial depends on whether the individual neurohormone is harmful or whether an elevated level of a beneficial neurohormone is an indication that homeostatic mechanisms are trying to oppose the effects of other potentially harmful mechanisms.

Taking the examples of norepinephrine and BNP, conventional wisdom is that a drug action to bring down plasma norepinephrine concentration is desirable. The desired action of a treatment on BNP concentration, however, is controversial. Some investigators like to see a fall in plasma BNP concentration with treatment, and might even titrate the dose of a treatment against the observed BNP response. (Murdoch, McDonagh et al. 1999) An alternative theory is that a drug that increases BNP concentration is beneficial by “releasing” a self-protective mechanism. The natriuretic peptide system is itself a homeostatic mechanism, with beneficial actions of high concentrations of BNP in the pathological state of CHF; the action of elevated BNP levels is to oppose vasoconstriction and fluid retention. Thus, raising BNP is actually desirable, even though the presence of a supra-normal level of BNP is a marker for the existence of CHF. Indeed, pharmacological measures have been taken both in acute (Publication Committee for the VMAC Investigators (Vasodilatation in the

Management of Acute CHF) 2002) and chronic heart failure (Packer, Califf et al. 2002) to increase BNP concentration as a means of therapy.

In interpreting the results of alterations to neurohumoral measures, one approach is to classify the measures themselves as being either harmful or helpful. For example, norepinephrine is harmful due to increasing heart rate, causing peripheral vasoconstriction, and generally increasing cardiac workload. B-type natriuretic peptide is helpful, causing vasodilatation and natriuresis.

In general, the effect of metoprolol in this study was to reduce the plasma concentrations of “harmful” neurohormones. This effect was consistent across the renin/ angiotensin/ aldosterone system (where renin, angiotensin II, and aldosterone were all reduced) and the sympathetic system (where plasma epinephrine was reduced, while plasma norepinephrine was not increased). Very little effect on endothelin was seen. Metoprolol treatment was also associated with raised levels of the “helpful” neurohormone BNP, but no effect was noted on ANP.

The action of carvedilol was not so clear-cut. Epinephrine and norepinephrine concentrations both increased with carvedilol. There was no effect on renin, a reduction in aldosterone, and an increase (non-significant) in angiotensin II. However carvedilol did increase both ANP and BNP to significant degrees. Endothelin level was unaffected. Taken together, the overall neurohumoral action of metoprolol would appear to be more beneficial than that of carvedilol. A possible reason for this difference would be that the vasodilating action of carvedilol performs some of the actions that would require the activation of protective neurohumoral mechanisms in metoprolol-treated patients.

There have been a number of previous publications examining the effects of metoprolol and carvedilol on various members of the panel of neurohormones we chose to examine in this study. However, these studies generally examine the effects of an individual beta-blocker on one or two members of the panel in isolation. The only other comparable study examining the action of carvedilol and metoprolol on components of the renin-angiotensin and natriuretic peptide systems differs somewhat from our findings.(Fung, Yu et al. 2003) This study found a short term (12 week) reduction in plasma renin activity and in aldosterone concentration, effects which disappeared at one year. Decreases in both natriuretic peptides were found.

In one of the largest neurohormone profile studies so far published, the placebo-controlled Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) pilot study (426 patients randomized), the effect of 24 weeks of metoprolol therapy on a broad panel of neurohormones was measured.(RESOLVD study investigators 2000) This study remains a reference for metoprolol's action, and it is of note that the actions seen were very similar to those in the metoprolol arm of our study.

The main difference between our study and RESOLVD is that in that study, 24 weeks of metoprolol therapy increased both plasma BNP *and* N-terminal ANP concentrations. Only one other small study, like ours comparing metoprolol and carvedilol, assesses the effect of carvedilol on natriuretic peptide concentrations.(Hirooka, Yasumura et al. 2001; Fung, Yu et al. 2003) In this study, a decrease in both N terminal BNP and in ANP was found, clearly at odds with our results and with those of RESOLVD. In one small uncontrolled study, metoprolol produced a decrease in BNP and ANP.(Hara, Hamada et al. 2000) In our study, both

BNP and N-terminal ANP were increased during therapy with carvedilol. Given the potentially favourable actions of natriuretic peptides in CHF, this action may contribute to the beneficial effects of beta-blockers in CHF.

In our hands, metoprolol also suppressed plasma renin concentration whereas carvedilol did not. This action of beta blockers to reduce plasma renin activity is well known.(Holmer, Hense et al. 1998) Previous studies, including RESOLVD, (RESOLVD study investigators 2000) have consistently demonstrated this action of metoprolol in CHF.(Jansson, Dahlstrom et al. 1999) Until recently, little was known about the effect of carvedilol on renin: another small study has now demonstrated an early significant reduction in plasma renin activity with both beta-blockers, but this reduction disappears after one year of treatment. (Fung, Yu et al. 2003)The relative lack of suppression of renin in the carvedilol group may also have reflected the increase in plasma norepinephrine with this treatment, as norepinephrine is known to elevate plasma renin. The changes in plasma angiotensin II concentrations tended to mimic those in plasma renin concentrations, though these were not significant for either beta-blocker.

Aldosterone was, therefore, a surprise, in that it appeared to be reduced similarly by both beta-blockers, but only significantly so by carvedilol. There is some support for this. (Fung, Yu et al. 2003) In the paper by Fung et al, the absolute reduction in aldosterone concentration at one year is considerably greater in the carvedilol group than in the metoprolol group. Aldosterone secretion is, however, controlled by factors other than angiotensin II and often does not necessarily change in parallel with the other components of the renin-angiotensin axis. (Foster, MacFarlane et al. 1997) The findings of RESOLVD are consistent with this view in that aldosterone was not

suppressed by metoprolol whereas both renin and angiotensin II were.(Krum, Gu et al.; 2000) Teisman et al performed a retrospective observation of chronic beta-blocker effects (in moderate to severe chronic heart failure with a mean treatment period of 3.8 years) and showed a similar tendency to reduce plasma aldosterone, though the effect did not reach statistical significance.(Teisman, van Veldhuisen et al. 2000) The literature contains no other reports of the effects of beta-blockers on aldosterone in CHF. One study in *hypertensive* patients showed that chronic carvedilol reduced plasma aldosterone concentration.(Dupont 1990) This suggests that the effect we observed with carvedilol may be a real one and may distinguish carvedilol from metoprolol.

While neither beta-blocker had a significant effect on epinephrine (as found in other studies), there was a differential action on norepinephrine. Plasma norepinephrine concentration increased significantly after carvedilol, whereas it did not with metoprolol. The existing literature on the effect of beta-blockers on norepinephrine in CHF is conflicting.(Swedberg, Hjalmarson et al. 1979; Nemanich, Veith et al. 1990) The largest neurohumoral study with metoprolol, RESOLVD, showed no significant difference from placebo.(RESOLVD study investigators 2000) We can find no well-controlled carvedilol-placebo comparisons. Other studies suggest that carvedilol either does not change or reduces circulating norepinephrine concentrations.(Gilbert, Abraham et al. 1996; Azevedo, Kubo et al. 2001)

Two studies show that carvedilol seems to reduce coronary sinus norepinephrine concentration. Together these two studies also suggest that carvedilol reduces cardiac and whole body norepinephrine release to a greater extent than metoprolol.(Gilbert, Abraham et al. 1996; Azevedo, Kubo et al. 2001) In one of these studies (an amalgam

of subjects from two larger studies with different entry criteria) there was a high proportion of patients with idiopathic dilated cardiomyopathy (IDC) and the baseline heart rates from the two groups differed significantly, suggesting different baseline characteristics.(Gilbert, Abraham et al. 1996) In the other study, no details are given about the underlying cause of CHF.(Azevedo, Kubo et al. 2001) As IDC has a different natural history than CHF due to ischaemic heart disease results from studies on these patients may well not be comparable to the patient group we studied.

In two further studies comparing carvedilol to metoprolol in CHF, there was either a trend to a reduction in venous plasma norepinephrine concentrations,(Kukin, Kalman et al. 1999) or a significant reduction,(Hirooka, Yasumura et al. 2001) with both carvedilol and metoprolol but no difference between treatment groups. There are however two studies in hypertensive patients that show that carvedilol increases plasma norepinephrine concentration.(Meyer-Sabellek, Schulte et al. 1985; Tomlinson, Smith et al. 1986) It is thus possible that carvedilol is more likely than metoprolol to increase plasma norepinephrine concentration, perhaps because of its additional alpha-adrenoreceptor blocking activity. For example, the selective $\alpha 1$ -adrenoreceptor antagonist prazosin increases norepinephrine in patients with CHF.(Colucci, Williams et al. 1980)

We found that plasma endothelin-1 (ET-1) concentration did not significantly change with either beta-blocker. Metoprolol did not reduce ET-1 in RESOLVD,(RESOLVD study investigators 2000) but there is one report that carvedilol does reduce plasma ET-1 concentration.(Krum, Gu et al.) This study, however, used very small numbers of patients (only 10 patients were treated with carvedilol, with 5 control patients). The larger retrospective observation of chronic beta-blocker effects mentioned above

revealed a tendency to reduce plasma endothelin though even in this larger group, as in our study, the effect did not reach statistical significance.(Teisman, van Veldhuisen et al. 2000)

As mentioned above, the findings from RESOLVD are largely in agreement with ours. Whilst we had no placebo group, the general change in the plasma concentrations of these neurohormones in patients treated with metoprolol was replicated in our study. Published data on the influence of carvedilol is more difficult to glean.

6.5. CONCLUSION

As drawn out in the haemodynamics chapter, the effect of metoprolol and carvedilol in beta-blocking terms was not the same in our study. However, this study is in general agreement with the reference RESOLVD study in its observation of the neurohumoral influence of metoprolol, and elements of other studies support most of the findings with carvedilol.

Definite differences exist in the actions of the two agents on neurohumoral systems. As the neurohumoral hypothesis is the cornerstone of our modern understanding of the pathophysiology of CHF, this raises difficulties in comprehending the actions of metoprolol and carvedilol in CHF. If neurohumoral influences are all-important in terms of prognosis, why is an almost identical benefit seen with both agents in large-scale trials? Perhaps the mode of action of both agents is actually quite different, though the magnitude of the end result on prognosis is similar.

CHAPTER 7: ANTIOXIDANT EFFECTS

7.1. OBJECTIVE

Carvedilol is known to have antioxidant properties *in vitro* and *in vivo*, (Yue, Cheng et al. 1992; Maggi, Marchesi et al. 1996; Nakamura, Kusano et al. 2002) and it has been postulated that this may be beneficial in CHF. No data exists to suggest that metoprolol is an antioxidant. Chronic heart failure is regarded as a condition in which an oxidative state exists, (McMurray, Chopra et al. 1993; Keith, Geranmayegan et al. 1998) and some observers suggest that this contributes to progression of the condition. A drug with antioxidant properties might therefore be advantageous in CHF.

We wished to compare the effects of chronic treatment with the two beta-blockers on a panel of measures of oxidant activity, to see if the *in vitro* antioxidant action of carvedilol is measurable *in vivo* and specifically to see if the effect is measurable in CHF patients. The antioxidant parameters are compared in CHF patients at baseline and after treatment with carvedilol or metoprolol for 10 and 20 weeks.

7.2. BACKGROUND

7.2.1. DEFINING OXIDATIVE STRESS

Many disease states ranging from aging to cancer and coronary heart disease have been associated with excess free-radical activity, or oxidative stress, and *antioxidants* have received attention as a potential therapy for these conditions. (Cross, Halliwell et al. 1987) A free radical is any molecule possessing an unpaired electron. In

biological systems the most important free radicals result from the addition of electrons to molecular oxygen.(Fridovich 1978)

Normally reduction of oxygen to water requires four electrons and occurs in mitochondria via the cellular respiratory chain: there is no production of reactive intermediates. However, univalent reduction of oxygen also takes place one electron at a time in a variety of physiologic as well as potentially pathologic processes, liberating partially reduced intermediates. These include free-radical superoxide anion, the non-radical hydrogen peroxide, and the highly reactive hydroxyl radical. These highly reactive species are referred to collectively as reactive oxygen species (ROS). Examples of ROS reactions with other biological molecules include the removal of electrons (oxidation) that can result in bond scission as well as the abstraction of hydrogen atoms. The resultant modification of organic molecules by ROS can be referred to as oxidative injury. Because of the reactivity of ROS, several enzymatic and non-enzymatic defenses such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), vitamin E, and vitamin C exist to protect against oxidative damage to other organic molecules.(Halliwell 1994) Oxidative stress, which may result in oxidative tissue damage, occurs when there is an imbalance between ROS production and antioxidant defenses such that either ROS production is increased and/or defense mechanisms are impaired.

7.2.2. OXIDATIVE STRESS AND CHF IN HUMANS

Based on extensive non-human evidence, the biological plausibility of the oxidative stress hypothesis of CHF is well established.(Dhalla, Hill et al. 1996; Hill and Singal

1997) In humans there are only a few specific disorders in which a clear link between oxidative stress and chronic ventricular dysfunction has been established: anthracycline-mediated cardiomyopathy,(Singal, Deally et al. 1987) alcoholic cardiomyopathy,(Edes, Toszegi et al. 1986) and prolonged selenium deficiency or Keshan disease.(Xu, Wang et al. 1997)

In these examples there are clear mechanisms that account for oxidative stress, either due to increased ROS generation, or impaired antioxidant defenses. However, whether free-radical processes have a pathophysiological role in the vast majority of patients with CHF due to other causes is unclear. No large observational epidemiological studies and prospective longitudinal cohort studies linking free-radical activity to CHF in humans exist. The evidence is limited to observational case-controlled studies that have demonstrated an association between markers of oxidative stress and clinical CHF.(McMurray, McLay et al. 1990; Belch, Bridges et al. 1991; McMurray, Chopra et al. 1993; Diaz-Velez, Garcia-Castineiras et al. 1996; Keith, Geranmayegan et al. 1998; Mak, Lehotay et al. 2000)

The reported studies for which appropriate control data are available are small and overall include about 200 patients with CHF. Aside from small sample size, limitations of many of these studies were confounding factors within the patient population. In some reports, evidence of oxidative stress was likely not specific to the heart failure state because of the presence of other conditions in the study population associated with oxidative stress, such as coronary artery disease or risk factors for atherosclerosis. In other studies, risk factors for atherosclerosis were not well characterized.

Diaz-Velez et al. compared patients with CHF to patients with cardiovascular risk factors and normal LV function as well as to healthy control subjects.(Diaz-Velez, Garcia-Castineiras et al. 1996) These investigators found no difference in markers of oxidative stress between the two patient groups, suggesting the risk factors themselves are associated with an oxidative state. Aging may also be associated with increased oxidative stress.(Stadtman and Berlett 1998) To address some of these issues, McMurray et al stratified patients with CHF by the presence or absence of coronary artery disease and found that, regardless of aetiology, CHF was associated with markers of oxidative stress when compared to age-matched control subjects.(McMurray, Chopra et al. 1993) CHF patients have also been compared to age-matched control subjects with normal LV function and found increased markers of oxidative stress in CHF despite the presence of atherosclerosis in both groups.(Mak, Lehotay et al. 2000) The available evidence that oxidative stress is *specific* to CHF in humans is, however, not yet conclusive.

7.2.3. BIOCHEMICAL MEASURES OF OXIDATIVE STRESS

There is no direct means to measure free-radical species in human *in vivo* studies, as they are highly reactive and short-lived. Thus human studies have quantified the *consequences* of free-radical reactions. The next two sections explain the two most widely used approaches, techniques which were employed in this project.

7.2.3.1 MEASURING LIPID PEROXIDATION

Polyunsaturated lipids are susceptible to free-radical attack, or “lipid peroxidation”. Stable decomposition products are produced, including aldehyde compounds that can then be measured in plasma as an indirect index of free-radical activity.(Gutteridge and Halliwell 1990) Malondialdehyde is one of many aldehyde compounds produced by lipid peroxidation and is frequently measured in plasma by the thiobarbituric acid-reactive substances (TBARS) assay. Thiobarbituric acid reacts with malondialdehyde to produce a stable compound that can be quantified using either spectrophotometry (as described in section 2.11.3) or high-performance liquid chromatography (HPLC).

7.2.3.2 MEASURING ANTIOXIDANT DEFENCES

Most investigators have attempted to quantify antioxidant defences as another means of detecting oxidative stress in humans.(Belch, Bridges et al. 1991; McMurray, Chopra et al. 1993; Keith, Geranmayegan et al. 1998) In patients with CHF, decreased thiol groups in plasma as well as those associated with erythrocyte membranes have been consistently demonstrated, (Belch, Bridges et al. 1991; McMurray, Chopra et al. 1993) possibly reflecting increased interaction between free radicals and membrane-associated proteins.

The activities of various antioxidant enzymes have been measured in plasma with inconsistent results. Reduced glutathione (GSH) as an important part of the antioxidant defense system by scavenging free radicals and regenerating other antioxidants. In CHF, both significant increases of plasma GSH and decreases of

whole-blood reduced GSH have been demonstrated.(McMurray, Chopra et al. 1993; Yucel, Aydogdu et al. 1998) The activity of erythrocyte SOD (eSOD) has been generally found to be depressed, although one report showed no difference in eSOD activity compared to control.(Nishiyama, Ikeda et al. 1998) The activity or serum concentrations of a variety of other antioxidants published in small reports include plasma ceruloplasmin (increased in CHF),(McMurray, Chopra et al. 1993) GPX (decreased in CHF)(Keith, Geranmayegan et al. 1998), vitamin E (not different from control in CHF)(Keith, Geranmayegan et al. 1998), and vitamin C (decreased and not different from control in CHF)(Keith, Geranmayegan et al. 1998). Thus, there is apparently little consensus regarding which plasma index of antioxidant status is most useful, and the data available have been inconsistent.(Mak and Newton 2001)

7.2.4. ASSESSMENT OF OXIDATIVE STRESS IN CHF: CONCLUSION

The existing evidence for a role of oxidative stress in the pathophysiology of CHF in humans is incomplete and measurement of free-radical activity *in vivo* in humans is not straightforward. Clinical trials of antioxidant therapy for CHF are few in number and, thus far, have failed to demonstrate convincing benefits.(Mak and Newton 2001)

7.3. RESULTS

7.3.1. EFFECTS ON LDL OXIDATION LAG TIME

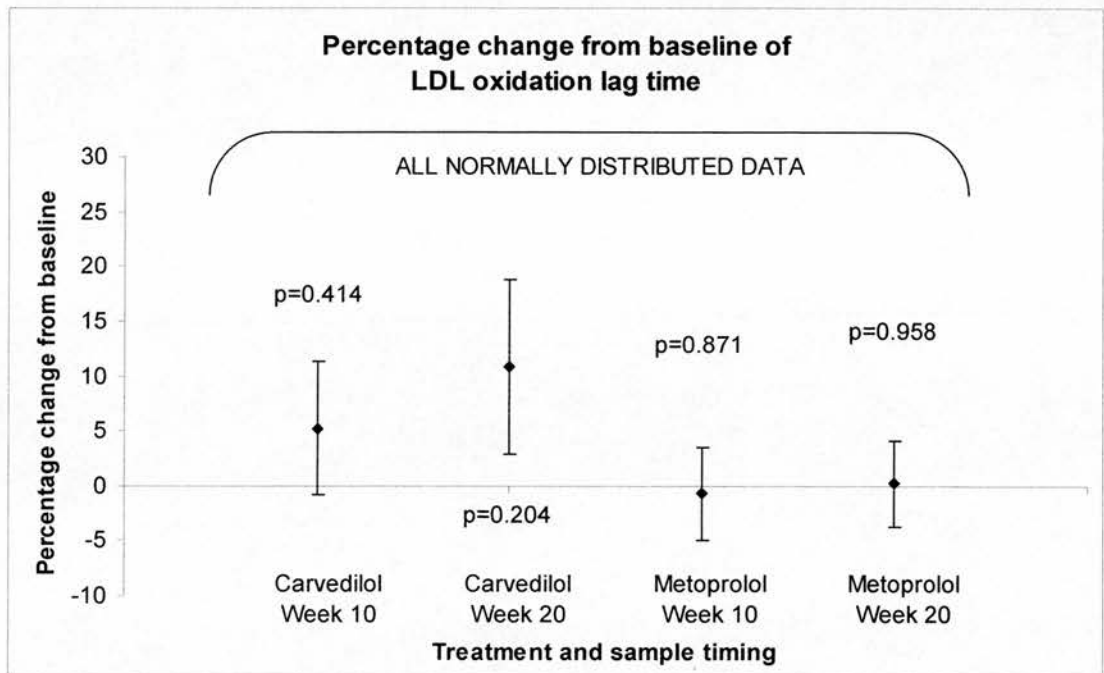
There was no statistically significant effect of either carvedilol or metoprolol to alter LDL oxidation lag time, though there was a slight effect of carvedilol to increase the LDL oxidation lag time (see Figure 7.1). The LDL oxidation lag time stayed almost exactly the same during treatment with metoprolol.

Table 7.1. Effect of treatment on LDL oxidation lag time.

LDL oxidation lag time					
Percentage change from baseline	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	P- value
	Carvedilol Week 10	10	5.23%	6.10%	0.414
	Carvedilol Week 20	10	10.89%	7.94%	0.204
	Metoprolol Week 10	10	-0.70 %	4.18%	0.871
	Metoprolol Week 20	10	0.21%	3.92%	0.958

Figure 7.1. Effect of treatment on LDL oxidation lag time.

The points represent means and bars represent standard errors.



7.3.2. EFFECTS ON PLASMA CONCENTRATION OF VITAMIN E

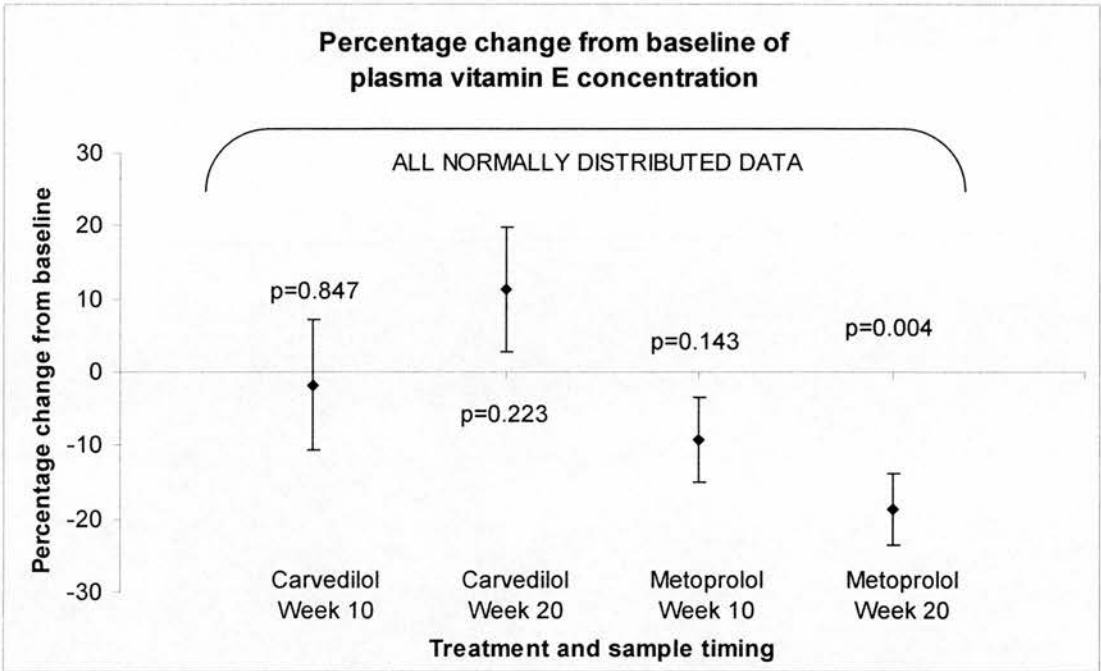
Vitamin E concentration increased slightly after 20 weeks of carvedilol therapy (though not after 10 weeks). Metoprolol did not stop the decrease in plasma vitamin E concentration, which was a non-significant effect at 10 weeks but became highly significant at 20 weeks of treatment (mean reduction of 18.7% from baseline, $p=0.004$) (see Figure 7.2).

Table 7.2 Effect of treatment on plasma vitamin E concentration.

Plasma Vitamin E concn.	NORMALLY DISTRIBUTED DATA (t-test)				
Percentage change from baseline	n	Mean	s.e.	P-value	
	10	-1.78%	8.94%	0.847	Carvedilol Week 10
	10	11.25%	8.59%	0.223	Carvedilol Week 20
	10	-9.25 %	5.76%	0.143	Metoprolol Week 10
	10	-18.66%	4.90%	0.004	Metoprolol Week 20

Figure 7.2. Effect of treatment on plasma Vitamin E concentration.

The points represent the means, the error bars represent the standard errors.



7.3.3. EFFECTS ON PLASMA MALONDIALDEHYDE CONCENTRATION

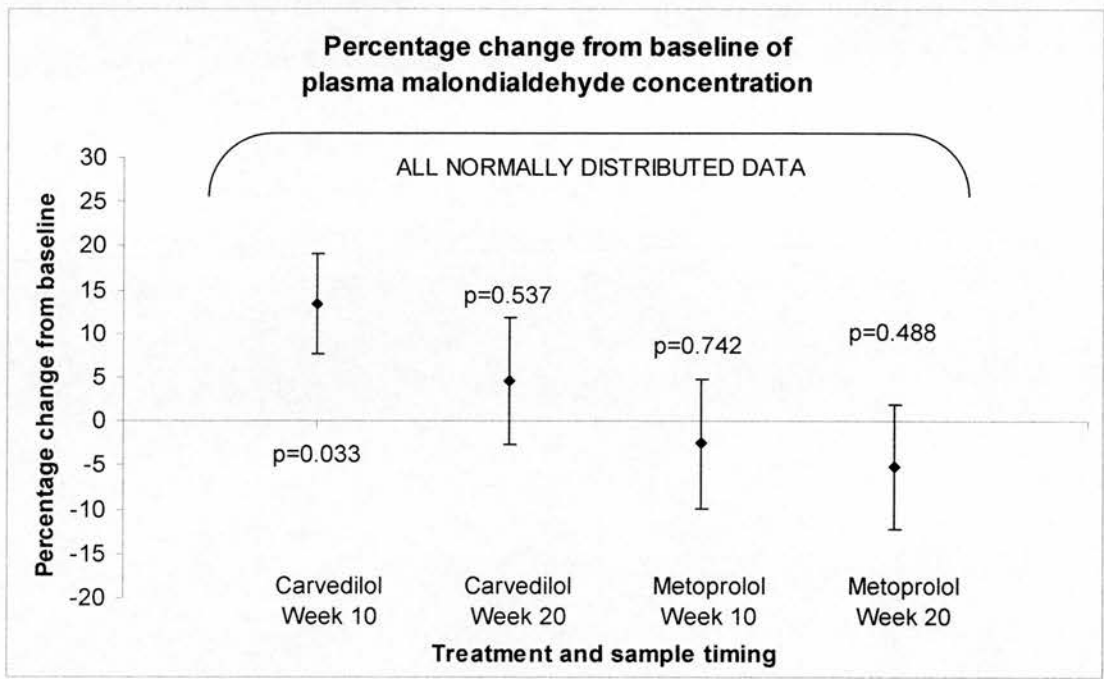
Malondialdehyde concentration was significantly increased by ten weeks treatment with carvedilol, but this effect disappeared after 20 weeks. Metoprolol had no significant effect on malondialdehyde concentration.

Table 7.3 Effect of treatment on plasma malondialdehyde concentration

Plasma malondialdehyde concentration					
Percentage change from baseline	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	P-value
	Carvedilol Week 10	17	13.42%	5.74%	0.033
	Carvedilol Week 20	17	4.60%	7.30%	0.537
	Metoprolol Week 10	12	-2.49 %	7.37%	0.742
	Metoprolol Week 20	12	-5.07%	7.06%	0.488

Figure 7.3. Effect of treatment on plasma malondialdehyde concentration

Points represent means, error bars represent standard errors.



7.3.4. EFFECTS ON TOTAL PLASMA ANTIOXIDANT ACTIVITY

Carvedilol also appeared to have a slight effect on total plasma antioxidant activity, increasing this parameter but again failing to reach statistical significance.

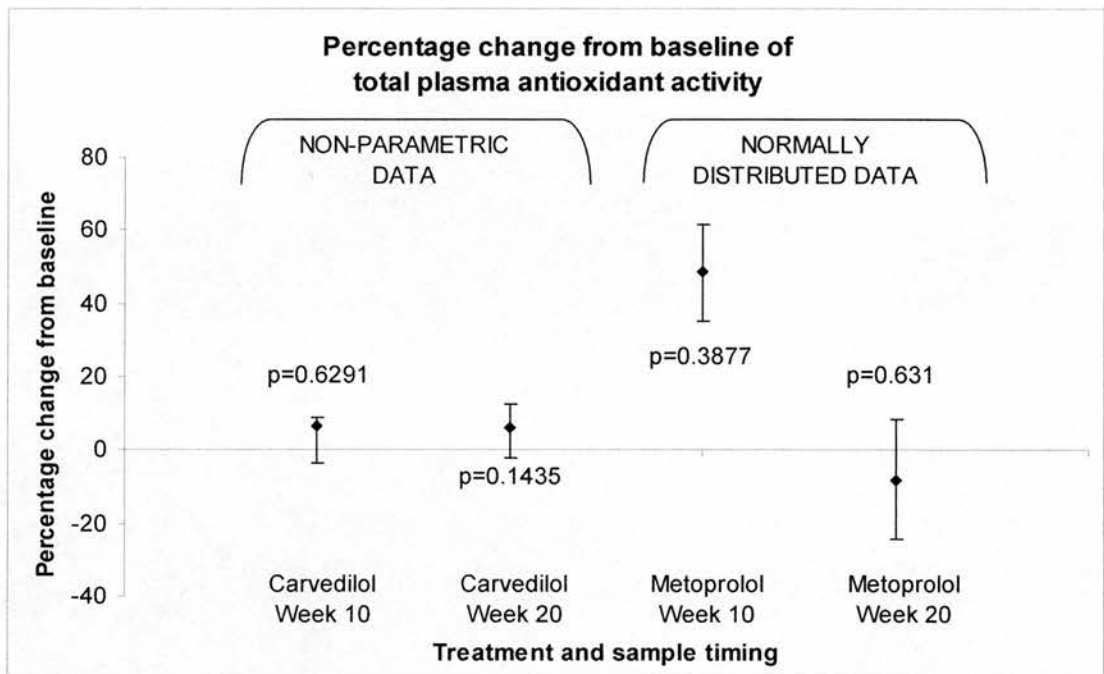
Metoprolol had no effect at 10 weeks, but very slightly (but not statistically significantly) increased total plasma antioxidant activity at 20 weeks (see Figure 7.4).

Table 7.4 Effect of treatment on total plasma antioxidant activity

Total plasma antioxidant activity						
Percentage change from baseline	NON-PARAMETRIC (1-sample sign test)					
		n	Median	95% CI (lower)	95% CI (upper)	P-value
	Carvedilol Week 10	17	6.50%	-3.56	9.11	0.6291
	Carvedilol Week 20	17	6.30%	-2.22	12.52	0.1435
	NORMALLY DISTRIBUTED DATA (t-test)					
		n	Mean	s.e.	T-value	P-value
	Metoprolol Week 10	12	48.53%	-13.2	192.9	0.3877
	Metoprolol Week 20	12	-8.1%	16.34%	0.49	0.631

Figure 7.4. Effect of treatment on total plasma antioxidant activity.

For normally distributed data, the points represent means and bars represent standard errors. For non-normally distributed data, the points represent medians and bars represent 95% confidence intervals.



7.4. DISCUSSION

Again, due to the small number of patients involved in this study, there was a large variance for some measures making it impossible to be certain that there is no difference between the groups in some measures. It is also possible some of the apparently statistically significant changes shown are spurious.

There were trends to increased resistance to LDL oxidation and increased total plasma anti-oxidant capacity in the carvedilol group that were not apparent in the metoprolol group. These were not, however, statistically significant. Conversely, plasma vitamin E concentrations fell in the metoprolol group (significantly so after 20 weeks treatment, consistent with consumption of this anti-oxidant) but not in the carvedilol group. Malondialdehyde concentration was significantly increased by 10 weeks treatment with carvedilol, going against its supposed action as an antioxidant.

Overall, our findings do not suggest that carvedilol has an anti-oxidant action *in vivo*. Kukin et al did not find any difference in anti-oxidant activity between carvedilol and metoprolol in CHF though only used plasma thiobarbituric acid-reactive substances (TBARS) as an indirect marker of lipid peroxidation.(Kukin, Kalman et al. 1999)

Other biochemical pathways and treatments can also influence TBARS.

Arumanayagam et al. noted a reduction in the results from assays of glutathione peroxidase and sodium dismutase with carvedilol (and no effect of metoprolol) in CHF patients, suggesting carvedilol might exert an anti-oxidant effect.(Arumanayagam, Chan et al. 2001) Total plasma anti-oxidant activity was not significantly reduced in this study, in keeping with our findings.

Our findings, and those of other studies, show that metoprolol is an effective anti-adrenergic drug as evidenced particularly by its effect to reduce heart rate (see section 4.4.1). The toxic effect of catecholamines *per se* may be important in the pathophysiology, and particularly the progression, of CHF.(Singal, Beamish et al. 1983; Obata and Yamanaka 1997; Qin, Rounds et al. 2001) As catecholamines exert some of their deleterious effects by oxidation, anti-oxidant agents would be expected to reduce some of this harmful effect. The only significant effect we were able to demonstrate with metoprolol was a reduction in vitamin E concentration, suggesting rather that metoprolol does little to reduce oxidation.

It could be that it is due to its anti-*oxidant* effect, rather than its anti-*adrenergic* effect that carvedilol has a part of its beneficial action. However, we were unable to confirm this effect here. There appeared to be trends for carvedilol to act as an antioxidant when assessed by LDL oxidation, vitamin E concentration and by total plasma antioxidant activity. However, the only statistically significant effect seen with carvedilol was a short-lived effect to *increase* malondialdehyde concentration, suggesting carvedilol did little to prevent oxidation at ten weeks.

7.5. CONCLUSION

Our findings suggest, but cannot confirm, that carvedilol has an anti-oxidant action *in vivo*, as well as *in vitro* when three out of four parameters are assessed. However, the carvedilol group increased ten-week malondialdehyde concentration, suggesting carvedilol was permitting an *increased* oxidative state at this time point. It is still

conceivable that the antioxidant action of carvedilol in the setting of CHF explains part of its beneficial clinical effect.

Metoprolol, despite being an anti-adrenergic drug only has a significant effect to permit an increase in plasma vitamin E concentration, suggesting it does nothing to reduce the oxidative state in CHF.

CHAPTER 8: CYTOKINES

8.1. OBJECTIVE

It has been recognised that chronic heart failure is associated with increased plasma cytokine concentrations,(Levine, Kalman et al. 1990) and it is suggested that the syndrome of heart failure is in part an inflammatory condition in which cytokines play a pathological role.(Mann and Young 1994; Anker and Rauchhaus 1999)

Cytokines tend to become elevated in patients with severe CHF, when cachexia is present.

We decided to investigate whether there were differential effects of carvedilol and metoprolol on the plasma concentrations of TNF- α and of its soluble receptor sTNFR-2 after 20 weeks of treatment.

8.2. BACKGROUND

Cytokines are low molecular weight proteins with both autocrine and paracrine actions. Unlike hormones, they are not stored but are secreted in response to specific stimuli. Tumour necrosis factor was first identified as a substance that exerted profound anti-tumour effects *in vitro* and *in vivo*. Besides its cytostatic and cytotoxic effects, TNF influences growth, differentiation and/or function of virtually every cell type studied, including cardiomyocytes. Elevated levels of TNF- α have been documented in the circulation(Levine, Kalman et al. 1990) and in the myocardium of patients with CHF.(Torre-Amione, Kapadia et al. 1996)

Nucleated cell types within the myocardium, including cardiomyocytes, synthesize TNF in response to noxious stimuli, including myocardial ischaemia/infarction and left ventricular pressure or volume overload. TNF does not exist in unstressed mammalian myocardium, but is rapidly expressed under conditions of stress. When the inciting stress is removed, levels return rapidly toward baseline. Expression of cytokines in response to stress can occur in the absence of immune system activation.

TNF- α is a 51 kiloDalton trimeric molecule with a half-life of 30 minutes. Its *plasma* concentration is most readily measured by an immunoreactive assay. TNF- α is known to promote left ventricular remodelling (Suffredini, Fromm et al. 1989) and is negatively inotropic (Torre-Amione, Kapadia et al. 1995). In advanced CHF, high levels of TNF- α are found in cachectic patients (McMurray, Abdullah et al. 1991; Anker, Chua et al. 1997). Animal experiments have shown that TNF- α is capable of inducing skeletal muscle wasting and apoptosis (Tracey, Morgello et al. 1990; Krown, Page et al. 1996) and the levels of TNF found in cachectic patients have been found to be the strongest predictors of the degree of weight loss in these patients. There is also an inverse relationship between serum level of TNF- α and peripheral blood flow (which is diminished in CHF) (Anker, Volterrani et al. 1998). These characteristics of TNF make it a candidate for producing some of the less well-understood clinical abnormalities present in advanced CHF.

The actions of TNF- α are mediated by binding to one of two specific receptors (TNFR-1 and TNFR-2), which are present on most cell types (Bolger and Anker 2000). The extracellular domain fragments of both receptors are shed from cell surfaces and can be detected as soluble forms (sTNFR-1 and sTNFR-2), the roles of

which are as yet unclear. At high concentrations, sTNFRs inhibit the activity of TNF- α and thus it has been proposed that they may be involved in regulation of immune status in CHF.

It is thought that high plasma concentrations of sTNFRs primarily indicate a history of raised TNF- α values. Elevated sTNFRs concentrations have been positively correlated with clinical class of CHF.(Keith, Geranmayegan et al. 1998) The reproducibility of plasma concentrations of sTNFRs is higher than that of TNF- α itself. This may be the reason why sTNFRs predict short term and long term prognosis better than TNF- α in CHF patients. Several untoward effects seem to be associated with raised TNF- α production in CHF.

At the time this study was conceived, sTNFR-2 was believed to be the most accurate cytokine predictor of prognosis in CHF, and hence its choice as our investigational marker.(Ferrari, Bachetti et al. 1995) Since then, an elevated level of sTNFR-1 has also been cited as the strongest and most accurate predictor of poor survival in CHF, independent of established parameters.(Rauchhaus, Doehner et al. 2000)

8.3. RESULTS

We were unable to demonstrate any significant influence of either of our two beta blocking agents on the plasma concentrations of TNF- α and sTNFR-2. As shown (in Table 8-1, Table 8-2, Figure 8-1 and Figure 8-2), analysis of the percentage change in plasma concentrations of TNF- α and sTNFR-2 (analysed in this way to correct for

differences in baseline concentrations between groups) did not show meaningful changes over the course of the study.

8.4. DISCUSSION

As with the other analyses, this study involved a small number of patients, so there was a large variance in all measures making it impossible to be certain that there is no difference between the groups.

As our study involved stable patients with CHF, baseline plasma concentrations of the two substances were not very high. None of our patients were, for instance, cachectic. Consequently, the likelihood of seeing a significant shift in the concentrations of either TNF- α or sTNFR-2 was not great. This may be the main reason that no change in cytokine concentrations could be demonstrated in this study.

It has been shown previously that metoprolol and bisoprolol can lower TNF- α in an uncontrolled study in patients with idiopathic dilated cardiomyopathy (IDC). (Ohtsuka, Hamada et al. 2001) A more recent study from the same group compares the effect of carvedilol and metoprolol on cytokine levels (TNF- α and interleukin-6) in idiopathic dilated cardiomyopathy. (Ohtsuka, Hamada et al. 2002) In this study, *both* drugs acted to reduce TNF- α over a 12-week period. The numbers of patients involved in the study were very similar to those involved in ours. Carvedilol differed from metoprolol in also reducing the concentration of interleukin-6. Clearly, these TNF- α results differ from ours.

This discrepancy may be due to the fact that our patients had different baseline characteristics (almost all of our patients had *ischaemic* heart failure as opposed to IDC), and the overall neurohumoral responses of our patients were different. Natriuretic peptide levels in our patients *increased*, whereas in the IDC study the plasma concentrations decreased. It is conceivable that these differences are due to the different underlying conditions, or due to differences in the severity of CHF in the two groups studied. Idiopathic dilated cardiomyopathy is, at least in theory, a much more “inflammatory” condition than ischaemic heart disease, and none of our patients were by definition in the early period after an ischaemic insult when any inflammatory process would be likely to be in progress. It is also possible that the changes in neurohormones and in cytokines seen in the IDC study reflected spontaneous improvement of the underlying condition amongst some subjects. This view is supported by a sub-study of MERIT-HF, a large placebo-controlled study with metoprolol, which showed no effect of metoprolol on cytokines.(Gullestad, Ueland et al. 2001)

Cytokines are known to have important interactions with neurohormones in CHF. In particular, elevated levels of noradrenaline and adrenaline act as catabolic stress hormones in advanced heart failure, and high levels are closely associated with high levels of inflammatory cytokines. Whilst some successful drugs reduce TNF,(Liu and Zhao 1999; Tsutamoto, Wada et al. 2000) a number of agents that successfully reduce cytokine levels cause increased mortality in CHF.(Matsumori, Shioi et al. 1994; Iwasaki, Matsumori et al. 1999) Indeed, recently there have been reports of the use of anti-cytokine drugs (being used to treat arthritis) resulting in new onset heart failure.(Kwon, Cote et al. 2003)

By protecting the myocardium from the action of catecholamines, beta-blockers are more likely simply protecting myocardium from part of the ongoing stimulus for the production of cytokines.

8.5. CONCLUSION

Our investigation reveals no significant effect of either carvedilol or metoprolol on plasma concentrations of TNF- α and sTNFR-2. There may be a slight trend for carvedilol to reduce TNF- α , but this is not statistically significant. It is possible that the stability of our patient population makes quantification of the effects of cytokine-modulating medication difficult.

Table 8-1 : Effect of treatment on plasma TNF- α concentration

TNF- α							
Percentage change from baseline	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	Absolute change (pg/ml)	s.e.	T-value	P-value
	Carvedilol Week 10	16	-6.6%	-1.34	1.34	7.63%	0.404
	Carvedilol Week 20	16	-12.1%	-2.48	2.48	12.65%	0.355
	Metoprolol Week 10	10	6.0%	1.41	1.41	12.2%	0.638
	NON-PARAMETRIC DATA (1-sample sign test)	n	Median		95% CI (lower)	95% CI (upper)	P-value
	Metoprolol Week 20	10	0.54%	0.55	-7.38	16.99	1.0000

Table 8-2 : Effect of treatment on plasma TNF receptor concentration

TNF-R2						
Percentage change from baseline	TNF-R2	NORMALLY-DISTRIBUTED DATA (t-test)	N	Mean % change	Absolute change (ng/ml)	P-value
		Carvedilol Week 10	17	1.5%	0.07	3.68%
		Carvedilol Week 20	17	-1.9%	-0.09	5.16%
		Metoprolol Week 10	12	-2.8%	-0.14	2.76%
		Metoprolol Week 20	12	2.3%	0.12	3.44%

Figure 8-1. Effect of treatment on plasma TNF-alpha concentration

For normally distributed data, the points represent means and bars represent standard errors. For non-normally distributed data, the points represent medians and bars represent 95% confidence intervals.

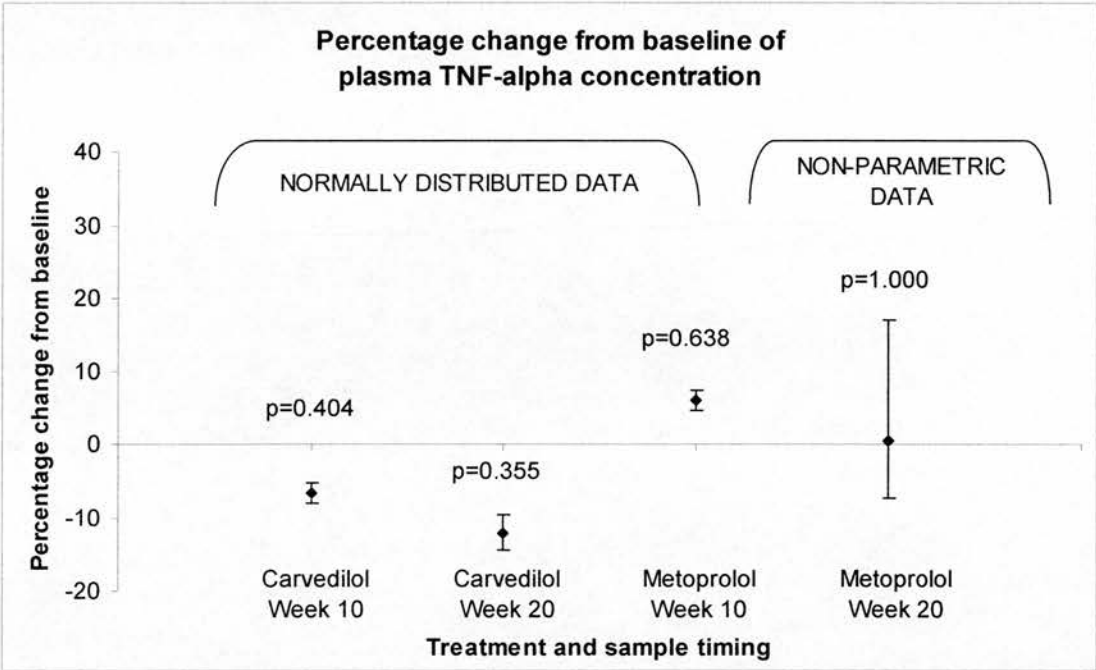
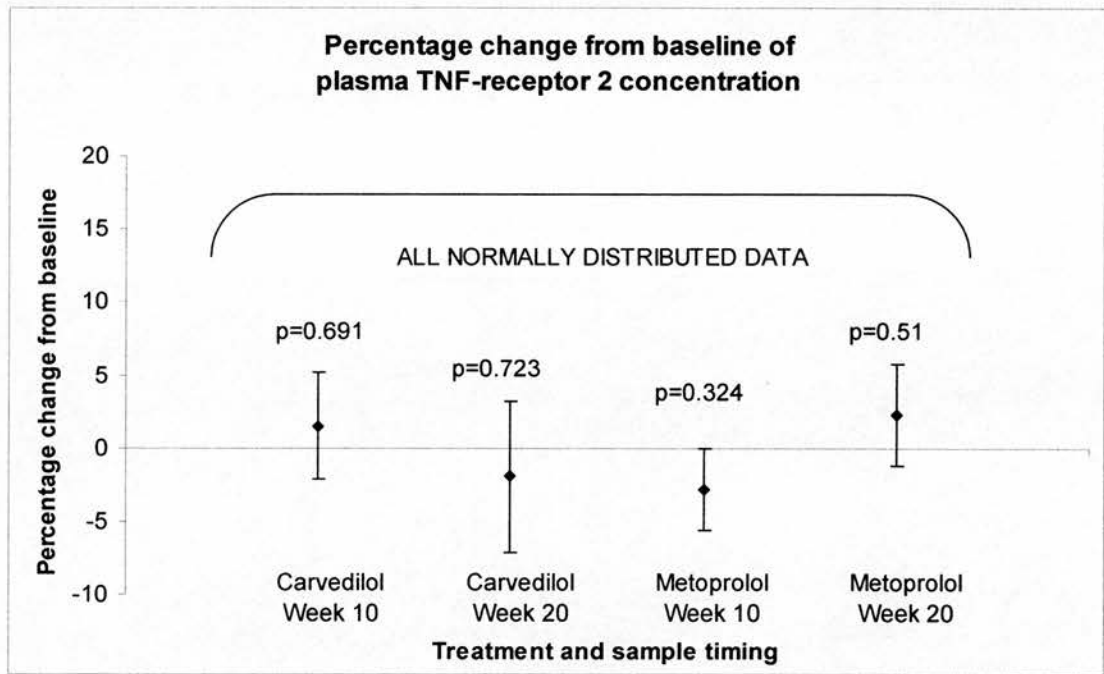


Figure 8-2. Effect of treatment on plasma TNF-R2 concentration.

Points represent means, bars represent standard errors.



CHAPTER 9: LYMPHOCYTE β RECEPTOR DENSITY

9.1. OBJECTIVE

Changes in beta-receptor density are an acknowledged feature of CHF.(Colucci, Alexander et al. 1981; Bristow, Ginsburg et al. 1986; Fowler, Laser et al. 1986) The influence of some beta-blockers on beta-receptor density has been investigated,(Heilbrunn, Shah et al. 1989; Yoshikawa, Handa et al. 1996) and differences are known to exist between beta-blockers with different receptor specificities.(Yoshikawa, Port et al. 1996)

Using carteolol, there is an early (2 week) effect to reduce *lymphocyte* beta receptor density which is abolished by 6 months of treatment.(Yoshikawa, Handa et al. 1996) Heilbrunn et al. (Heilbrunn, Shah et al. 1989)showed that treatment with metoprolol leads to receptor up-regulation in *myocardial* tissue, whilst Yoshikawa et al.(Yoshikawa, Port et al. 1996) showed that carvedilol does not lead to this up-regulation.

In this part of the study, we sought to confirm whether differences exist between the influence of metoprolol and carvedilol on lymphocyte beta-receptor density. As it is difficult to directly measure the beta-receptor density on *cardiomyocytes* (the invasive technique of myocardial biopsy is required), lymphocyte beta-receptor density has often been used as a surrogate measure.(Colucci, Alexander et al. 1981; Yoshikawa, Handa et al. 1996) A difference in the effect of the two agents on this parameter would suggest a mechanism by which metoprolol and carvedilol vary in their action on beta-receptors in cardiomyocytes.

9.2. BACKGROUND

When the heart fails, chronic sympathetic activation results in selective down-regulation of β_1 adrenoreceptors and uncoupling of both β_1 and β_2 adrenoreceptors from G proteins, markedly blunting β -adrenergic receptor signalling.(Bristow 2000) In end-stage heart failure, 50%-60% of the total signal-transducing potential is lost, but substantial signalling capacity remains. We now speculate that these changes are in fact adaptive, as the heart tries to reduce the harmful effects of adrenergic overactivity.

9.2.1. MECHANISM OF NOREPINEPHRINE CARDIOTOXICITY

There are 3 well-characterised adrenergic receptors in human cardiomyocytes (β_1, β_2 and α_1) coupled to a positive inotropic response and cell growth.(Bristow, Ginsburg et al. 1986; Brodde, Schuler et al. 1986; Bristow 1993) These β -Adrenoreceptors are coupled via the stimulatory G-protein G_s to adenylate cyclase, activation of which results in the production of cyclic AMP. This is a positive inotrope and chronotrope, and is strongly growth promoting. In non-failing human ventricles, the β_1/β_2 ratio is 70-80/ 20-30, but in failing ventricles the number of β_2 adrenoreceptors increases to 35-40% because of selective downregulation of the β_1 adrenoreceptor subtype.(Bristow, Ginsburg et al. 1986; Brodde, Schuler et al. 1986) The G-protein G_q couples α_1 -adrenoreceptors to the effector enzyme phospholipase C and diacyl glycerol is produced on activation of this system. Diacyl glycerol activates the growth-promoting protein kinase C family. As α_1 -adrenoreceptors are up-regulated

in heart failure, the cardiomyocyte profile changes from predominantly β_1 to a more mixed 2:1:1 ratio in end stage heart failure.(Bristow 1993)

Norepinephrine is highly cardiotoxic,(Mann, Kent et al. 1992) producing cardiomyocyte injury at concentrations found in failing human heart. It is mildly β_1 selective (10- to 30-fold compared to the affinity to β_2 receptors), and its cytotoxicity appears to be mediated through β rather than α -adrenergic receptors.(Mann, Kent et al. 1992) In transgenic mice, cardiac over expression of human β_1 receptors(Port, Weinberger et al. 1998; Engelhardt, Hein et al. 1999) or of the stimulatory G proteins G_{α_s} (Iwase, Bishop et al. 1996)or G_{α_q} produces an overtly cardiomyopathic phenotype,(D'Angelo, Sakata et al. 1997) resulting in chamber dilatation and systolic dysfunction. Over expression of the stimulatory G protein G_{α_s} is also associated with increased markers of apoptosis, which can be produced in adult rat cardiomyocytes by β agonist exposure.(Bristow 2000) In adult rat cardiomyocytes, the β_1 receptor mediates apoptotic signalling, whereas the β_2 receptor is anti-apoptotic via coupling to the inhibitor protein, G_i .(Communal, Singh et al. 1999) High levels of cardiac over expression of the human β_2 receptor eventually result in depressed systolic function and a cardiomyopathic phenotype,(Liggett, Tepe et al. 2000) and cardiac expression of a constitutively activated α_1 receptor produces concentric hypertrophy.(Milano, Dolber et al. 1994) Thus data from model systems indicate that chronic adrenergic signalling, especially β_1 receptor signalling, is a harmful compensatory mechanism in the failing human heart.

9.2.2. MECHANISM OF DOWN REGULATION OF BETA RECEPTORS

Families of regulator and adaptor molecules control the amplitude, duration and targeting of adrenergic receptor signals and are intimately involved in the blunting of β -adrenergic receptor signalling. There are many mechanisms for turning off signals transduced by G-protein-coupled receptors. Members of the G-protein-coupled receptor kinase family (GRK1 up to GRK6) phosphorylate adrenergic receptors when occupied by an agonist. This phosphorylation increases the affinity of adrenergic receptors for cytosolic proteins, known as β -arrestins, which prevent further G-protein coupling. The β -arrestins also act as scaffold molecules, coupling the adrenergic receptors to different downstream effectors. In end-stage heart failure, 50 to 60% of the total signal transducing potential is lost, but substantial signalling capacity remains:(Bristow 1993) the suggestion is that the β -adrenergic receptor desensitisation seen is adaptive, so an effective therapeutic strategy is to add to this action.

Key to Figure 9.2

Mechanism of down-regulation of beta adrenoreceptor sensitivity in CHF

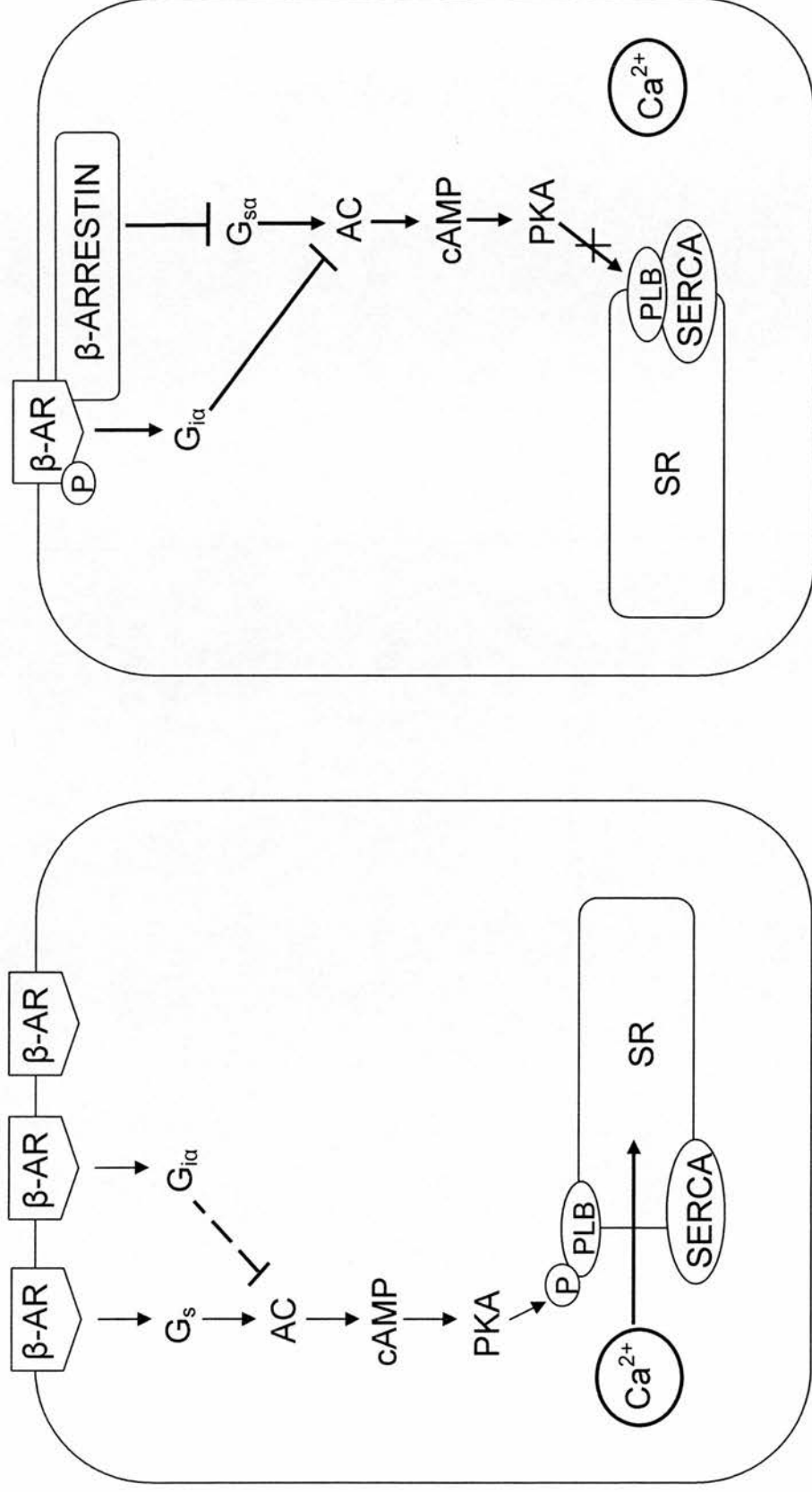
NORMAL HEART:

High membrane density of β -adrenoceptors (β -ARs). Stimulatory G proteins (G_{α}) activate adenylate cyclase (AC), raising cAMP levels to induce protein kinase A (PKA). Activated PKA phosphorylates phospholamban (PLB), releasing its inhibitory function on the sarcoplasmic reticulum (SR) calcium ATPase pump (SERCA). This enhances Ca^{2+} transport from the cytosol to the SR. The removal of Ca^{2+} from the cytosol augments relaxation, and adequate release of Ca^{2+} from the SR stores increases contractility.

HEART FAILURE:

Down-regulation of β -ARs (mainly the β_1 -AR subtype) and desensitization by phosphorylation occurs, impairing activation of G_{α} . β -arrestin couples to the receptor, uncoupling of the β -AR from G_{α} . There is also upregulation of the inhibitory G protein ($G_{i\alpha}$) and receptor internalization. Together, these mechanisms inhibit the β -adrenergic–PKA pathway, and the impaired Ca^{2+} cycling decreases contractility and relaxation.

Figure 9.2 Mechanism of down-regulation of beta adrenoreceptor sensitivity in CHF



9.3. RESULTS

Administration of neither metoprolol nor carvedilol produced a statistically significant change in the lymphocyte beta-receptor density after 10 or 20 weeks. The effect of metoprolol just failed to reach significance ($p=0.06$) at 20 weeks, with a *reduction* in density of 13.9% (see Figure 9.2 and Table 9.1).

9.4. DISCUSSION

9.4.1. TIME COURSE OF EFFECTS

During the week prior to screening assessments, the dose of beta-blocker therapy was halved and then stopped immediately before the first dose of study medication. This decision, taken at an early stage of study design, was intended to permit recruitment of patients who had been treated with beta blockers for indications other than CHF, such as angina, previous myocardial infarction or hypertension. In the group of patients studied, there was a high prevalence of these comorbidities. Without this decision it would have been extremely unlikely that recruitment could have been completed in the population available to the study. The rationale for the short reduction in dose and then removal of any prior beta blockade was to minimise any potential risk to these patients.

The consequences of the prior use of beta blockers may well have been important with regard to eventual study results and in particular the effect on the measured lymphocyte beta receptor density. Unfortunately this aspect of the study design may

have obscured the true effects of the study drugs. Whilst the short term action of the beta blockers may no longer have been present by the time of starting the study drugs, it is very likely that some residual alteration in beta adrenoreceptor density and function caused by earlier beta blocker treatment was present at the time of initiation of the study drugs. The receptor desensitisation which accompanies CHF and the change in beta receptor density requires fundamental changes in intracellular mechanisms, including receptor synthesis. Previous studies have investigated the time-course of change in beta adrenoreceptor function, though mainly in relation to the β_2 adrenoreceptor, and found that the effect of an agonist (presumably similar to the effect of removal of an antagonist) took up to 2 weeks to exert its maximum effect on beta adrenoreceptor density.(Bruck, Leineweber et al. 2003) A similar two week period was noted by another group investigating the effects of beta blocker on lymphocyte beta receptor density.(Yoshikawa, Handa et al. 1996) Thus it may well be that the time period used for withdrawal of the prior beta blocker was too brief and the reduction in dose should have been a complete withdrawal. Due to this unfortunate aspect of study design, the measured effects of the study drugs on beta adrenoreceptor density are difficult to decipher.

9.4.2. INFLUENCE OF LYMPHOCYTE VARIATION

A considerable body of evidence points to the modulation of immune responses by the autonomic nervous system.(Baerwald, Burmester et al. 2000) Lymphoid organs are densely innervated by noradrenergic neurones,(Felten, Felten et al. 1985) and as mentioned lymphocytes express mainly β_2 adrenergic receptors.(Brodde, Engel et al.

1981) However, there is great variability in the numbers of β_2 adrenergic receptors between lymphocyte subpopulations. CD8+ T cells, B cells and natural killer cells express more β_2 adrenergic receptors than do CD4+ T cells.(Landmann, Burgisser et al. 1984; Van Tits, Michel et al. 1990) As the proportions of these differing sybtypes are influenced by many other factors (such as the presence of asthma or other immune-mediated conditions, exposure to allergen, treatment with asthma medication such as beta agonists, or intense sympathetic nervous system activation), it is likely that the very blunt tool of measuring overall lymphocyte beta receptor density was a poor surrogate for cardiomyocyte receptor density. Other work has shown considerable variation between and within individuals in β adreneroreceptor density.(Anstead, Hunt et al. 1998) Despite this further major caveat, the use of peripheral blood lymphocyte beta receptor density as an alternative to endomyocardial biopsy has continued to be used by some groups even to the present day, with reported results which appear to speak to the value of the assessment.(Hata, Williams et al. 2006)

9.4.3. OTHER LIMITATIONS

As this study involved a small number of patients, there was a large variance for some measures making it impossible to be certain that there is no difference between the groups in some measures. Despite the lack of a statistically significant change, observation of the raw data does appear to show a tendency for carvedilol to increase beta receptor density, whilst the effect of metoprolol in either direction is not marked, with a possible reduction noted.

This component of the study has other definite limitations. Although it has been postulated that peripheral lymphocyte beta receptors may reflect alterations in beta receptor density and overall beta adrenergic responsiveness of less accessible tissue such as heart, human lymphocytes contain few if any β_1 receptors and are therefore not a reliable surrogate system for the heart.(Brodde, Michel et al. 1989) The measurements made, therefore, can be presumed to be of the effect of the agents primarily on β_2 receptors. For the purposes of this study, an effect of metoprolol would not be anticipated, whilst one might reasonably expect to see some change in receptor density in carvedilol-treated patients.

Lymphocyte beta receptors are not innervated, which thus leads to a lack of concordance between behaviour of myocardial and lymphocyte beta receptors in disease conditions manifested by increased adrenergic neurotransmitter activity, or during β_1 receptor specific therapeutic interventions.

As the predominant change in myocardial beta-receptor density is the decrease in β_1 receptors, it is debatable whether measurement of lymphocyte beta-receptor density is really valid.(Brodde, Michel et al. 1989) One of the effects of end-stage heart failure, as stated above, is for the density of myocardial β_2 receptors to increase. Carvedilol, whilst not having a statistically significant effect, does tend towards increasing lymphocyte beta-receptor density (and therefore β_2 receptors). It is not known whether or not this is a favourable influence.

It has previously been clearly shown that administration of metoprolol results in an increase in myocardial beta-receptor density, which results in a return of beta-

adrenergic sensitivity.(Heilbrunn, Shah et al. 1989) Based on current understanding, this is indeed a favourable influence of the agent. Whether a similar action of carvedilol occurs has unfortunately not been adequately addressed in this study.

9.5. CONCLUSION

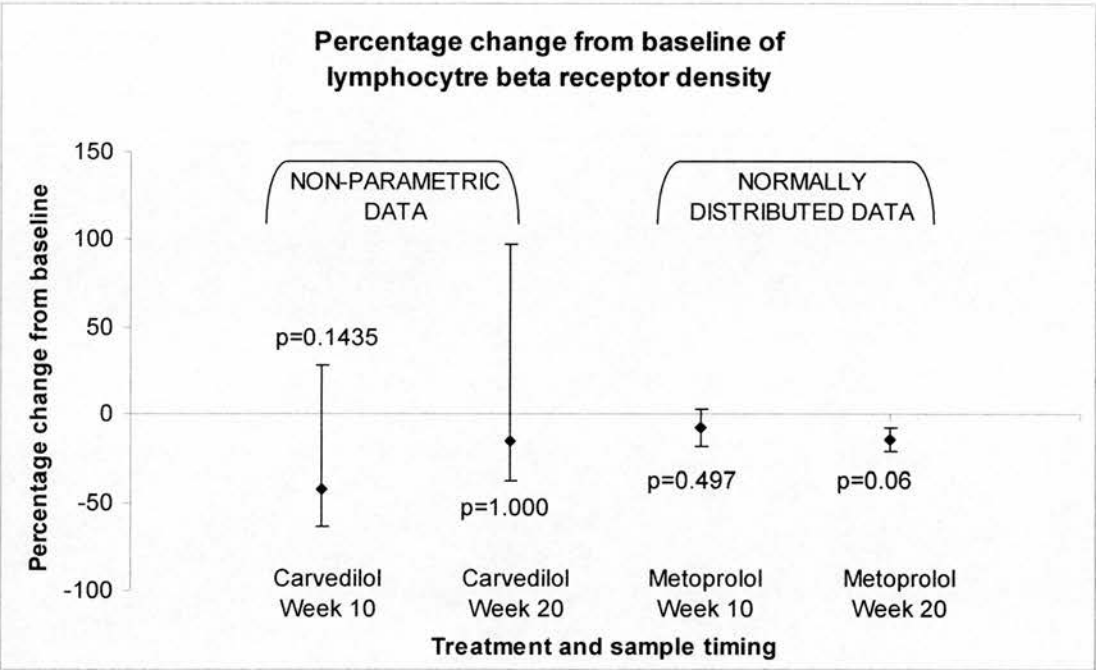
No significant differences appear to exist in the effects of metoprolol and carvedilol on lymphocyte beta-receptor density. There is an apparent trend for carvedilol to increase β_2 receptor density on lymphocytes, which could represent a real action of the drug to up-regulate β_2 receptors.

Table 9.1: Effect of treatment on lymphocyte beta receptor density

Lymphocyte beta receptor density						
Percentage change from baseline	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95%CI (upper)	P- value
	Carvedilol Week 10	17	-42.51%	-63.36	28.86	0.1435
	Carvedilol Week 20	17	-14.73%	-37.05	97.34	1.0000
	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	P-value	
	Metoprolol Week 10	11	-7.4%	10.52%	0.497	
	Metoprolol Week 20	11	-13.9%	6.57%	0.060	

Figure 9.2. Effect of treatment on lymphocyte beta receptor density

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.



CHAPTER 10: CARDIAC ELECTRICAL STABILITY

10.1. OBJECTIVE

Approximately 50% of CHF sufferers die of sudden cardiac death, which is believed to be due to ventricular arrhythmias in most cases.(Narang, Cleland et al. 1996)

Patients with CHF may be predisposed to the development of arrhythmia when an underlying inhomogeneity of myocardial repolarisation exists. As CHF progresses, the reduction in number and function of β_1 receptors means that the importance of β_2 receptors increases. Some data suggests that it is particularly antagonism at the β_2 receptors which protects against arrhythmia.(Billman, Castillo et al. 1997)

We wished to examine the effects of treatment with carvedilol and metoprolol on markers of cardiac electrical stability (signal averaged ECG and QT dispersion).

Both of these measures have been shown to have prognostic importance in patients with heart failure and in patients following myocardial infarction.(McClements and Adgey 1993; Somberg and Molnar 2002) We wanted to know if the two investigational drugs had differential effects on these parameters, suggesting that differences in the pharmacological properties of the drugs might have clinically important consequences on cardiac electrical stability.

10.1.1.1 QT DISPERSION: BACKGROUND

QTc dispersion is a surrogate marker of the inhomogeneity of myocardial repolarisation and predicts arrhythmias and sudden death in those with ischaemic heart disease, hypertrophic cardiomyopathy, long QT syndrome, CHF and other

conditions.(Somberg and Molnar 2002) Clinical and electrophysiological studies have shown the importance of inhomogeneous myocardial repolarisation characteristics in the genesis of ventricular arrhythmias. Increased dispersion of repolarisation provides a substrate for ventricular arrhythmias by generating areas of functional unidirectional block, thereby predisposing to reentry which appears to be the major mechanism by which ventricular arrhythmia occurs. (Zipes and Wellens 1998)

QT dispersion in the 12-lead ECG, defined as the difference between the longest and the shortest QT interval,(Cowan, Yusoff et al. 1988) is a validated measure of dispersion of repolarisation.(Somberg and Molnar 2002) It reflects regional differences in myocardial repolarisation and provides indirect evidence of arrhythmogenicity.

Increased QT dispersion and corrected QT dispersion have been reported in patients with CHF compared to controls, and are considered as potential markers for predicting drug effects on mortality.(Bonnar, Davie et al. 1999) A strong association of increased QT dispersion with sudden cardiac death has been identified.(Barr, Naas et al. 1994)

A number of trials of beta-blockers have shown that the incidence of sudden cardiac death is reduced during treatment with beta-blockers, most notably in CIBIS-II.(CIBIS-II investigators and committees 1999) Beta-blocker therapy in CHF has also been shown to reduce QT dispersion.(Bonnar, Davie et al. 1999; Fesmire, Marcoux et al. 1999) Our intention was to determine if QT dispersion is affected to different degrees by the two investigational drugs, metoprolol and carvedilol.

10.1.1.2 QT DISPERSION: METHODS

After resting to allow settling of any skeletal muscle activity, all patients had ECG recordings made at the same paper speed and gain setting (25 mm/sec and 10 mm/mV) using a Megacart ECG machine (Siemens, UK). Standard 12-lead ECGs were recorded with leads attached in the conventional positions. The usual exclusions for QT dispersion studies were already present due to the overall study exclusion criteria.

A single observer (SM) digitised all ECGs using a digitising board (Calcomp, Anaheim, California, USA) connected to an IBM-compatible personal computer and running customised QT dispersion software (Academic Cardiology Unit, Freeman Hospital, Newcastle upon Tyne). QT interval was measured from the beginning of the QRS complex to the end of the T wave, defined as the return to T-P baseline. When U waves were present, the QT interval was measured to the nadir of the curve between the T and U waves. QT intervals were measured in all leads if technically possible. For each lead, two or three consecutive cycles were measured and the arithmetic mean of the QT interval for that lead was used in all future calculations for QT dispersion. The measured values were then expressed as both uncorrected and rate-corrected QT intervals (QT and QTc).

10.1.1.3 SIGNAL AVERAGED ECG: BACKGROUND

The signal-averaged ECG was initially developed to allow recording of low-amplitude ventricular depolarisations on the surface ECG of myocardial infarct patients who are susceptible to ventricular tachycardia.(Kuchar, Thorburn et al. 1987; Farrell, Bashir et al. 1991) Although first used in this setting, the SAECG has since been applied to many different groups of patients.(Gomes, Cain et al. 2001) The link between ventricular “late potentials” recorded in the SAECG and re-entrant ventricular tachycardia in a wide variety of conditions is well established. (Breithardt, Cain et al. 1991)

Late potentials represent slow or delayed conduction originating from viable cells within or surrounding myocardial infarct regions, and are believed to represent a fixed substrate for re-entrant ventricular tachycardia. They are defined as abnormal signals, which outlast the normal QRS period during normal sinus rhythm.(Breithardt, Cain et al. 1991)

The noise in conventional ECGs ranges from 8 to 10 μV and is generated primarily by skeletal muscle, masking the late potentials from myocardial activity which one would wish to measure. The purpose of signal averaging is to improve the signal-to-noise ratio to facilitate the detection of these low-amplitude potentials. Temporal averaging is used in most commercial systems, reducing random or uncorrelated noise by the square root of the number of waveforms averaged. This process requires the existence of a repetitive and invariable potential of interest, with a fixed relationship to an easily detectable reference point such as the peak of the QRS complex. The signal of interest and the noise must be independent and remain

independent during averaging. Most signal processing systems use time-domain analysis to detect late potentials in the terminal QRS complex, requiring high-gain amplification and appropriate digital filtering to reject low frequencies associated with the plateau and repolarisation phases of the action potential, ST segment and T wave. This enhances detection of the high frequency signals that correspond to ventricular activation.(Cain, Anderson et al. 1996)

Orthogonal bipolar XYZ ECG leads are recorded, averaged, automatically filtered by software on the recording system, and combined into a vector magnitude called the filtered QRS complex. Analysis of the filtered QRS complex typically includes:

1. The filtered QRS duration (QRS)
2. The root-mean-square of the terminal 40 milliseconds of the filtered QRS (RMS)
3. The length of time that the filtered QRS complex remains $<40 \mu\text{V}$ i.e. the low amplitude signal duration (LAS)

Values of these measurements are dependent on the high pass corner frequency, which in our case was a 40-Hertz high-pass filter. Characteristics of a late potential (assuming use of a 40 Hertz high-pass bi-directional filter) include:(Cain, Anderson et al. 1996)

1. A filtered QRS complex duration >114 milliseconds
2. A signal $<20\mu\text{V}$ in the last 40 milliseconds of the filtered QRS complex
3. Voltage $<40\mu\text{V}$ in the terminal QRS complex for >38 milliseconds

10.1.1.4 SIGNAL AVERAGED ECG: METHODS

A Megacart ECG machine (Siemens, UK) with signal averaged ECG (SAECG) recording software installed was used to record all SAECGs. SAECGs were recorded immediately after the 12 lead ECG recordings as described above.

10.2. RESULTS

Seventeen patients in the carvedilol group and twelve patients in the metoprolol group had complete QT dispersion and saECG data suitable for analysis.

There was no significant change in QT dispersion (corrected for rate) noted with either agent (see Table 10-1, Figure 10-1). Turning to SAECG parameters, there was a slight prolongation of QRS duration with both agents as might be expected with beta-blocker treatment (see Table 10-2 and Figure 10-2) No significant difference was noted in the RMS with carvedilol (see Table 10-3 and Figure 10-3). Metoprolol had a much larger effect in comparison with carvedilol, and this effect was statistically significant ($p=0.011$). LAS duration increased in both groups to a very similar degree (see Table 10-4 and Figure 10-4). The effect on LAS in the metoprolol group was statistically significant ($p=0.026$), though the effect was not significant in the carvedilol group.

10.3. DISCUSSION

Due to the small number of patients involved in this study, it is possible the apparently statistically significant changes shown are spurious. As with the other analyses, this study involved a small number of patients, so there was a large variance for some measures making it impossible to be certain that there is no difference between the groups in these measures.

Serious ventricular arrhythmia is well recognised as a common mode of death in CHF. In several of the large-scale beta-blocker studies sudden cardiac death, believed to be due to ventricular arrhythmia in most cases, was an end-point that was particularly susceptible to the influence of beta-blockers.(Chadda, Goldstein et al. 1986; Packer, Bristow et al. 1996; 1999; 1999; Hjalmarson and Fagerberg 2000)

In fact, beta blockers have been shown to reduce the risk of sudden cardiac death in more than 50 randomised trials involving more than 55000 patients.(Hjalmarson 1999) Beta-blockers are well known to raise the threshold for ventricular fibrillation,(Parker, Michael et al. 1990) and are effective in suppressing ventricular tachycardia. Our chosen surrogate measures of cardiac electrical stability have both undergone rigorous examinations. The effect of metoprolol on LAS and RMS differentiated the two agents under study.

It has been shown that QT dispersion is susceptible to variation, with even time of day having an influence.(Smetana, Batchvarov et al. 2003) There is no doubt that QT dispersion is inherently difficult to measure in a reproducible way, and in particular the decision as to where the QT interval actually ends has been problematic.(Murray,

McLaughlin et al. 1994) This is the primary reason for measurement error in QT dispersion determination.(Somberg and Molnar 2002) Other observers, using a modification of QT dispersion which uses a longer recording (QT variability index) claim to have been able to identify a difference in the effects of carvedilol and metoprolol, with carvedilol producing a greater improvement on this parameter than metoprolol.(Piccirillo, Quaglione et al. 2002) The longer recording method in this study is likely to result in a more reproducible result.

As metoprolol was seen to be so much more effective at heart rate reduction (see section 4.4.1), which is usually the most readily assessed method of determining beta-blocking efficacy, one would have perhaps expected a more marked influence of this drug on markers of cardiac electrical stability. It is likely that the effect on LAS and RMS is some reflection of this. This more potent effect of metoprolol is again, like the effect seen on heart rate in this study, unexpected. The broader anti-adrenergic properties of carvedilol would be anticipated to suppress ventricular arrhythmia with greater potency than would metoprolol. Indeed, previous work has suggested that as far as altering the threshold for ventricular fibrillation is concerned, it is action on the β_2 receptor which matters.(Billman, Castillo et al. 1997)

10.4. CONCLUSION

Data from large clinical studies confirms the effect of beta-blockers to reduce sudden cardiac death. The surrogate measures used in this study were able to demonstrate a statistically significant change in some parameters after 20 weeks of treatment with metoprolol.

The techniques themselves have a number of methodological problems. It is likely that the effect on LAS and RMS seen with metoprolol reflects more the unexpected greater effect on heart rate reduction seen with metoprolol, rather than any true influence on electrical stability. Indeed, it is in fact surprising that *any* significant difference between the agents was detectable, particularly as an attempt was being made to discriminate between two inherently rather similar medications.

Table10-1: Effect of treatment on corrected QT dispersion

Lymphocyte beta receptor density						
Percentage change from baseline	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95%CI (upper)	P- value
	Carvedilol Week 20	17	-13.69	-33.56	9.93	0.1435
	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	P-value	
	Metoprolol Week 20	12	7.9 %	16.3%	0.637	

Table 10-2 : Effect of treatment on SAECG QRS duration

SAECG QRS duration						
Percentage change from baseline	NON-PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95%CI (upper)	P- value
	Carvedilol Week 20	17	1.020%	-3.000	4.208	0.6072
	NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	P-value	
	Metoprolol Week 20	12	2.4%	2.13%	0.280	

Table 10-3 : Effect of treatment on SAEKG RMS

SAEKG RMS duration					
Percentage change from baseline	ALL NORMALLY DISTRIBUTED DATA (t-test)	n	Mean	s.e.	P- value
	Carvedilol Week 20	17	-4.8%	6.54%	0.473
	Metoprolol Week 20	12	- 24.1%	7.3%	0.130

Table 10-4 : Effect of treatment on SAEKG LAS duration

SAEKG LAS duration						
Percentage change from baseline	ALL NON- PARAMETRIC DATA (1-sample sign test)	n	Median	95% CI (lower)	95% CI (upper)	P- value
	Carvedilol Week 20	17	3.85%	-1.95%	18.28	0.4545
	Metoprolol Week 20	12	- 113.02 %	- 141.62 %	-84.15	0.0005

Figure 10-1: Effect of treatment on percentage change in corrected QT dispersion

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.

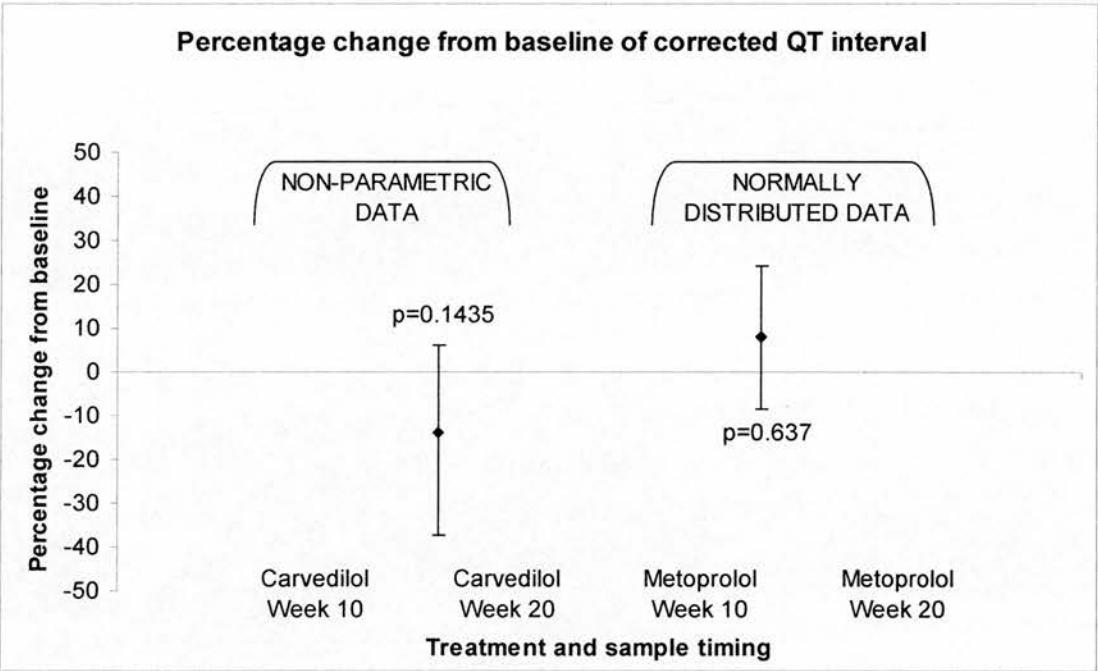


Figure 10-2 : Effect of treatment on percentage change of SAEKG QRS duration from baseline

For normally distributed data, the points represent means and bars represent standard errors. For non-parametric data, the points represent medians and bars represent 95% confidence intervals.

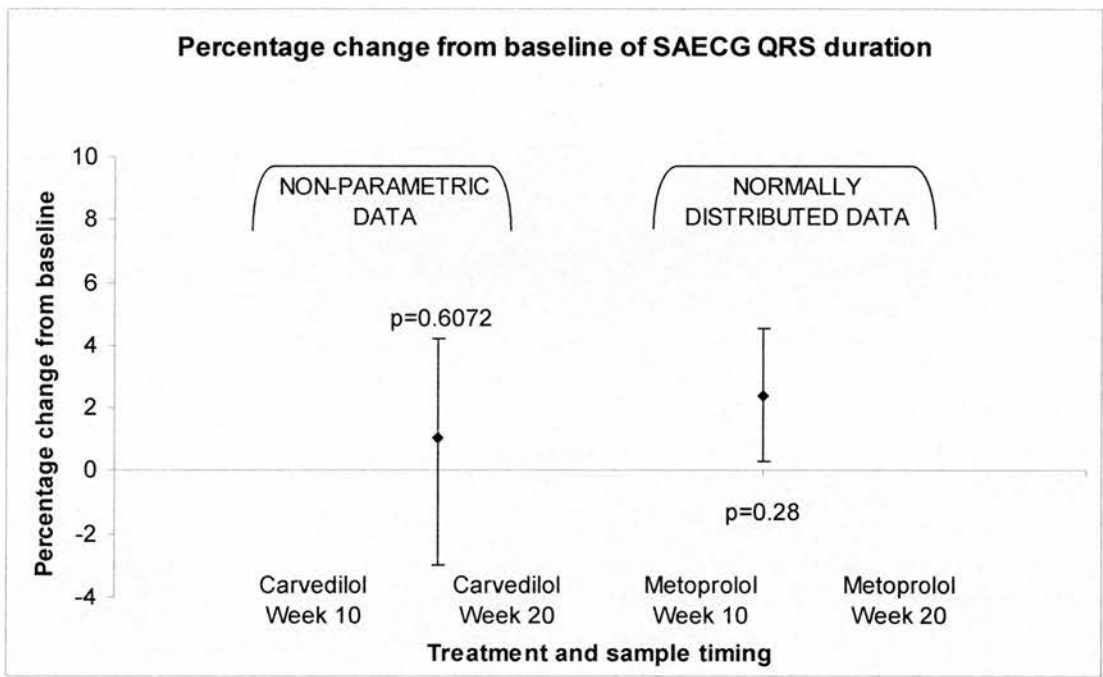


Figure 10-3 : Effect of treatment on SAEKG RMS percentage change from baseline

The points represent means and bars represent standard errors.

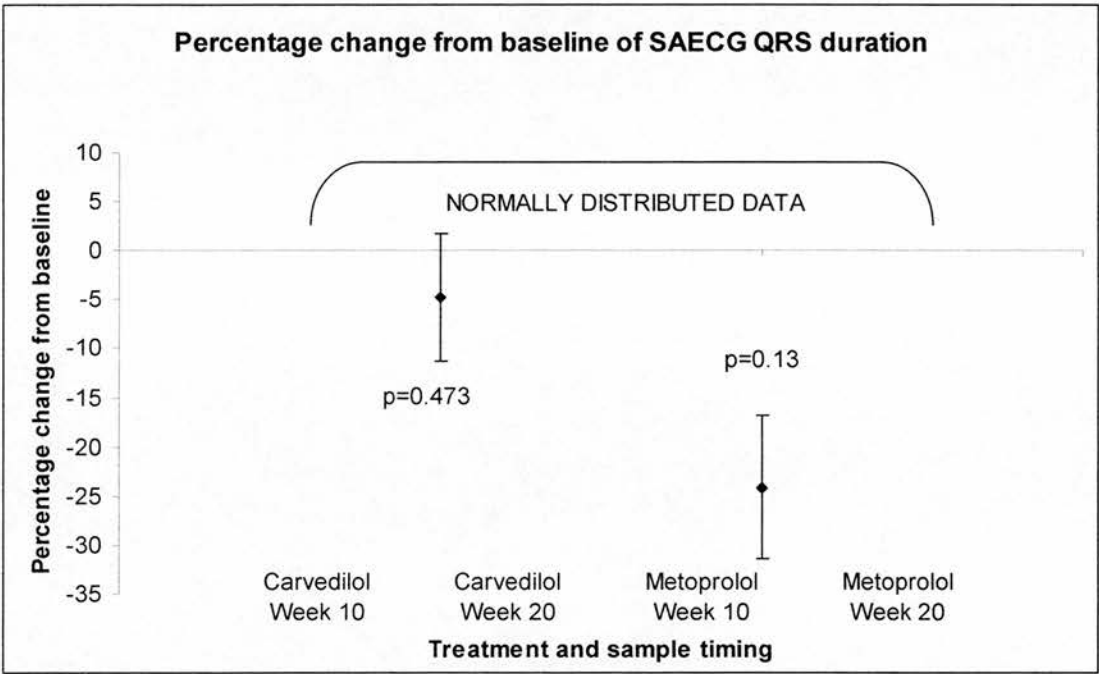
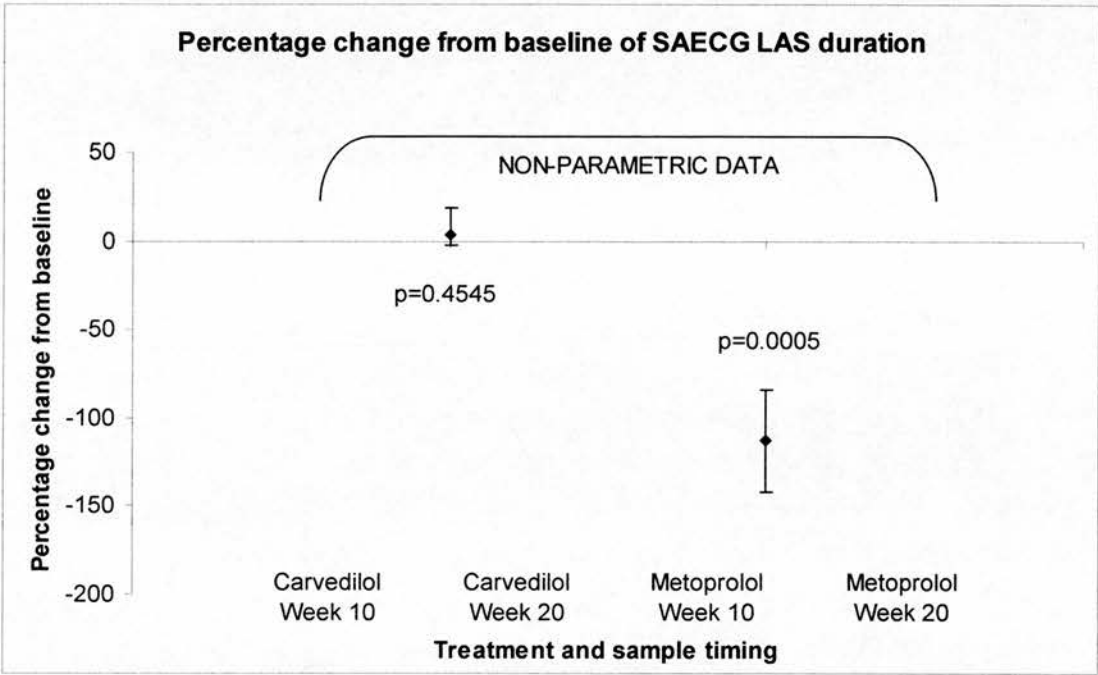


Figure 10-4 : Effect of treatment on SAECG LAS duration percentage change from baseline

The points represent medians and bars represent 95% confidence intervals.



CHAPTER 11: CONCLUSION

11.1. SUMMARY OF FINDINGS

We found that metoprolol and carvedilol reduced resting heart rate to a similar degree in the doses used. Metoprolol had a greater effect to suppress the increase in heart rate in response to beta agonism. Carvedilol had a much more widespread influence on the peripheral action of beta agonists than did metoprolol. Metoprolol generally resulted in more improvement in neurohumoral measures than carvedilol, whilst there was a trend for carvedilol to have an antioxidant action (though this fell short of reaching clinical significance). Metoprolol had a beneficial effect on some parameters of cardiac electrical stability. The effect of metoprolol on neurohumoral measures and on cardiac electrical stability may reflect the marginally greater effect this drug had on heart rate.

11.2. LIMITATIONS

11.2.1. STUDY DESIGN

In the design of this study, several problems arose. The most important of these was the timing of systemic infusions and peripheral arterial assessments with respect to the first dose of study medication. On the first intensive study day, the patients had already been treated with the first dose of study medication *prior to* systemic infusions and peripheral arterial assessments. Thus a true baseline for these parameters was not recorded. Whilst the first dose of study drug used was very small indeed (metoprolol 1.25mg, carvedilol 3.125mg), and would be unlikely to have had any measurable effect, it is scientifically unsatisfactory that this situation arose. This was unavoidable due to constraints by ethical considerations (see section 2.3). The

West Ethical Committee of Greater Glasgow Health board felt that it was unacceptable to involve patients in more than two of the day-long studies, and would also be unacceptable to involve the patients in more than two forearm plethysmographic studies which involve arterial cannulation. The committee also advised an overall reduction in the number of patient visits. We had complete sympathy with their views, as there is no doubt that involvement in four full days of study would have been a considerable undertaking for any patient, particularly those with significant CHF.

However, the restriction of the study design to permit a total of nine visits (including the screening visit) was to have major implications for the overall study. At the time the study was conceived, clinical practice (based on experience from the US carvedilol study) was to have up-titration visits at 2-weekly intervals. Thus the advice from the West Ethics Committee forced the combination of the first day-long plethysmographic study day with the first day of drug administration and meant that no true baseline was available for the effects of the drugs on forearm venous occlusion plethysmography nor for the responses to systemically-administered adrenergic agonists; this was the only way in which the number of visits necessary for supervised up-titration could be accommodated. In retrospect, it is deeply regrettable that this flawed study design was executed, at least without an appeal to the committee.

Our original study plan had intended the use of a true baseline study day, a study day just after initiation of medication, a study day after the maintenance dose had been reached, and a final study day. A table is shown below to illustrate the differences

between our “ideal” study structure and the actual protocol we eventually employed. We felt that our original study structure had greater scientific validity, but we were obviously compelled to abide by advice from the West Ethical Committee. The significant complexity of our study did not lend itself well to the use of two simple plethysmographic study days; one would speculate that that the West Ethical Committee decision was based on their experience with other simpler studies where the effect of a drug could more adequately be assessed by a simple “before and after” study design. The known nature of the action of carvedilol, with the anticipated waning of the α -blocking effect, makes more frequent assessment with plethysmography and dissection of the different adrenoreceptor actions desirable.

It is also very unfortunate that the intended number of plethysmographic assessments was not completed, due to problems arising in an unrelated study (see section 2.10.3). This greatly reduced the numbers of recordings performed.

11.2.2. IDEAL STUDY DESIGN

With the benefit of hind sight, it is now easy to appreciate some of the other flaws inherent in this study.

Certain of the techniques employed are easy to implement, such as blood analyses or ECG recording (for saECG or QT dispersion). To achieve more meaningful results would in the case of many of these parameters have required a much larger sample size as is obvious from the degree of variance in the results. Thus it would have been better to have sampled from a larger group of patients for these parameters.

Other techniques, especially forearm venous occlusion plethysmography, are much more difficult and time-consuming to employ and would have better been employed on a small subgroup. The more precise and reproducible nature of these assessments means less need for a large sample size in any case.

Table 11.1 Ideal study schedule

Key: AV=additional visit (did not occur in study)

ASSESSMENT	INITIATION AND TITRATION PERIOD										MAINTENANCE		Follow up
	Screening day (-7 to -1)	AV	Day 0	AV	WEEK 2	WEEK 4	WEEK 6	WEEK 8	WEEK 10	AV	WEEK 20		
Consent	X												
Medical history	X												
Concomitant medication check	X		X		X	X	X	X	X		X	X	
Cardiopulmonary examination	X		X		X	X	X	X	X		X		
Vital signs (HR,BP)	X		X		X	X	X	X	X		X	X	
Weight	X				X	X	X	X	X		X		
ECG+saECG	X										X		
NYHA class assessment	X				X	X	X	X	X		X	X	
Antioxidant tests	X								X		X		
Neuroendocrine tests	X								X		X		
Lymphocyte beta receptor density	X								X		X		
TNF- α and TNF- α soluble receptors	X								X		X		
Adrenergic effects (intra-arterial infusions +intravenous infusions)		X		X						X	X		
Bioimpedance (Cardiac output) monitoring		X		X						X	X	X	
FBF following occlusion	X										X		
Routine blood tests: FBC, renal function	X								X		X		
Adverse events assessment			X		X	X	X	X	X		X	X	
Compliance assessment			X		X	X	X	X	X		X		

11.2.3. POPULATION

This was of necessity a small study, and the size of population studied reduces the certainty with which results can be stated.

The patient population enrolled seems not to have been suffering from the same severity of CHF as other studies. Whilst the patients had medical histories, ECGs, echocardiographic data and clinical features (particularly their symptoms) in keeping with NYHA grade II-III CHF (see section 2.8), the low resting heart rate measured suggests that their CHF was very well controlled. It is certainly possible that the patients were clinically better than those enrolled in the large studies.

11.2.4. METHODOLOGY

In some areas, the methodology employed in this now seems inappropriate.

11.2.4.1 HAEMODYNAMICS

Whilst incontrovertible data was gleaned on most of the haemodynamic measures (heart rate, blood pressure), some authors regard non-invasive cardiac output monitoring as inferior to invasive means of assessing these parameters.(Barry, Mallick et al. 1997) Thus the data obtained on cardiac output and on stroke volume may not be robust. It would however have been very difficult, and likely ethically impossible, to have performed these assessments invasively.

11.2.4.2 PLETHYSMOGRAPHY

Forearm venous occlusion plethysmography is an elegant technique, and produced data which, whilst initially surprising to this author (see section 5.5.1), has corroboration in the literature.(Metra, Nodari et al. 2002)The main issue here is lack of numbers for the reasons stated above.

11.2.4.3 NEUROHUMORAL, ANTIOXIDANT AND CYTOKINE MEASURES

Most of these measures are well studied and are appropriate. Measurement of plasma epinephrine concentration was probably unnecessary, as it is notoriously susceptible to fluctuations.

As discussed in the antioxidant chapter (section 7.2), great doubts remain as to the validity of these measures, and in particular there are doubts about the validity of these measures for assessing response to therapy.(Mak and Newton 2001)

As our study patients all had relatively well-controlled CHF, and none were cachectic, the utility of cytokine assessments in this population must be questioned. It is most unlikely that any measurable influence of beta-blockers on cytokines would have been detectable in this population.

11.2.4.4 LYMPHOCYTE BETA RECEPTOR DENSITY

This parameter has been discussed in detail in section 9.4. The best and possibly the only truly relevant way of assessing myocardial beta-receptor density would have

been with myocardial biopsies, which would again have presented major ethical difficulties.

11.2.4.5 CARDIAC ELECTRICAL STABILITY

The main criticism here is use of relatively small numbers, and the attempt to compare two drugs which are inherently rather similar. This makes the likelihood of identifying a difference low. There is probably also a criticism that our patients were all relatively well, and would not have been expected to have very abnormal baseline parameters as assessed by these techniques.

11.2.5. β_3 ADRENORECEPTOR SUBTYPE

Over the period during which this study was conducted, the β_3 adrenoreceptor subtype was identified. The atypical effect of isoproterenol, a nonselective β -adrenoceptor agonist, on human ventricular endomyocardial biopsies in the presence of nadolol, a β_1 - and β_2 -adrenoceptor antagonist, suggested the presence of a functional third β -adrenoceptor subtype in human ventricular muscle.(Gauthier, Tavernier et al. 1996) Contrary to β_1 - and β_2 -adrenoceptor pathways, activation of this receptor by norepinephrine in the presence of α -, β_1 - and β_2 -antagonists, or by BRL 37344, a selective β_3 -adrenoceptor agonist, decreases contractile force.(Gauthier, Tavernier et al. 1996; Gauthier, Leblais et al. 1998) Initially identified in fat tissue(Krief, Lonnqvist et al. 1993) and subsequently in vascular

tissue(Gauthier, Langin et al. 2000), this receptor subtype has also been myocardium.(Gauthier, Tavernier et al. 1996; Gauthier, Leblais et al. 1998; Kitamura, Onishi et al. 2000; Cheng, Zhang et al. 2001; Moniotte, Kobzik et al. 2001)

In contrast to β_1 and β_2 adrenoreceptors, β_3 adrenoreceptors are linked to inhibitory G_i proteins, and stimulation of β_3 adrenoreceptors inhibits cardiac contraction and relaxation.(Gauthier, Leblais et al. 1998; Gauthier, Langin et al. 2000; Kitamura, Onishi et al. 2000; Cheng, Zhang et al. 2001; Moniotte, Kobzik et al. 2001)

Overexpression of human β_3 adrenoreceptors in mice reproduces ex vivo the negative inotropic effects.(Tavernier, Toumaniantz et al. 2003) Although the precise physiological and pathophysiologic roles of β_3 adrenoreceptors remain uncertain, some work suggests that in the normal heart, β_3 adrenoreceptors participate in nitric oxide (NO)-mediated negative feedback control over contractility.(Gauthier, Leblais et al. 1998) Data also suggest that following prolonged activation by the sympathetic nervous system the β_3 adrenoceptor-mediated response is likely to be preserved while the β_1 - and β_2 -adrenoceptor-mediated responses are attenuated. In addition to an increase in receptor abundance in CHF,(Moniotte, Kobzik et al. 2001) the evidence supports the view that the β_3 -adrenoceptor plays a dominant role in regulating cardiomyocyte function during the high adrenergic tone that is typical of heart failure.(Moniotte and Balligand 2002) Some recent evidence suggests that endogenous β_3 -adrenoceptor activation contributes to cardiomyocyte dysfunction in heart failure.(Morimoto, Hasegawa et al. 2004)

The β_3 adrenoreceptor is of obvious interest in the context of this study, as it is certainly possible that the drugs under study have differing effects on this new receptor. Unfortunately, as the existence and function of this receptor subtype was still under early investigation it was not possible to assess this potential.

11.3. CLINICAL IMPLICATIONS

The study reported in this thesis is obviously small, but was necessarily so in order that the mechanism of action of the two agents could be assessed in greater depth.

Whilst mechanism of action of the two drugs is obviously of great interest, particularly with future therapies in mind, it is in the end the clinical effect of the two agents is what will determine which one will be used.

11.3.1. THE COMET STUDY

The COMET study was intended as the definitive exploration of differences in clinical end-point between therapy with carvedilol and metoprolol.(Poole-Wilson, Cleland et al. 2002) This multi-centre, randomised, double-blind parallel-group trial compared the effect on mortality and morbidity of carvedilol and metoprolol in patients with chronic heart failure.(Poole-Wilson, Swedberg et al. 2003) COMET has one of the longest follow-up periods and greatest number of patient years of exposure of any heart failure trial. Of the 3029 randomised patients, 1511 on carvedilol were eventually assessed for primary end-points, while 1518 on metoprolol -were assessed for primary end-points. The mean study duration was 58 months. There were 512 deaths (438 cardiovascular) in the carvedilol group and 600 (534 cardiovascular) in

the metoprolol group. There was a 17% reduction in mortality in the carvedilol group relative to metoprolol ($p < 0.0017$, 95% confidence interval 0.74-0.93). The authors concluded that carvedilol had a significantly greater effect on survival than metoprolol.

The COMET study has far from ended the controversy over whether carvedilol or metoprolol is the superior beta-blocker in CHF. There is dispute over the dose and formulation of metoprolol used. MERIT-HF study employed an extended-release form of metoprolol succinate (metoprolol CR/XL), whilst COMET used more conventional metoprolol tartrate 50 mg BD. A dose of 100mg metoprolol tartrate per day was planned in COMET (actual mean dose was 85 mg). The mean dose of metoprolol in MERIT-HF was 159 mg of the CR/XL (succinate) formulation, equivalent to about 106 mg of metoprolol tartrate given the lower bioavailability of the succinate. In addition, the design of COMET had actually been based on metoprolol doses used in the MDC trial, where the mean dose achieved was 108mg metoprolol tartrate per day. (Waagstein, Bristow et al. 1993) Despite basing the regime on MDC, COMET administered metoprolol tartrate only twice a day, whereas in MDC metoprolol tartrate was administered three times a day. Thus the dose of metoprolol actually used in COMET was less than that shown to be effective in MERIT-HF, was less than that used in the MDC trial upon which COMET had based its dosing target. The fact that the metoprolol, despite being the short-acting tartrate form, was only given twice a day, compounds the issue.

This problem of equivalent dosing becomes even more obvious when observing the heart rate reduction in the metoprolol arm of COMET. Reductions in heart rate seen

in the first few months of COMET were 13.3 bpm in the carvedilol group and 11.7 bpm in the metoprolol group, though after longer treatment the heart rate reduction in both COMET arms was virtually identical.(Poole-Wilson, Swedberg et al. 2003) This effect on heart rate during the first few months in COMET leaves open the possibility that some of the difference in efficacy seen was due to differing degrees of beta blockade, simply due to an inadequate or at least non-equivalent dose of metoprolol. In addition, in MERIT-HF, using metoprolol succinate, the heart rate reduction was greater than in the metoprolol arm of COMET (14.0 bpm). This produced an annual mortality rate of 7.2% in MERT-HF compared to 10% in the metoprolol arm of COMET, despite recruitment of potentially a higher risk group of patients. It is difficult to conclude that in COMET, metoprolol exerted a similar degree of β_1 blockade as carvedilol.

11.3.2. COMPARISON OF COMET WITH THIS STUDY

The relative effects of the two drugs in COMET was the converse of the relative effect on resting heart rate that was seen in our study, though a statistically significant level of difference was not reached. In addition, our data suggests a more potent effect by metoprolol to limit the increase in heart rate with both β_1 and β_2 agonism. It is gratifying to note that the doses used in COMET were the same as in our study, where the mean daily dose of metoprolol was 91.7 mg. As with our study, COMET aimed for comparable reductions in resting heart rate within the two groups.

The effects of the two agents on heart rate in COMET, given the numbers involved in the study, make a compelling argument that carvedilol *is* the more potent at reducing heart rate when compared to twice daily metoprolol tartrate. Why this was not seen in our study could be due to:

1. different severity of heart failure in the two groups in this study
2. different sensitivity of the two groups to the actions of the beta blockers in general.
3. timing of measures of heart rate being more susceptible to a high peak dose of metoprolol compared to a more even dose of the longer-acting carvedilol.

The first explanation is unlikely, as resting heart rates and other features were very similar (see Table 2.3 and Table 3.1).

The second explanation is more plausible. There are known polymorphisms of beta-receptor subtypes, which result in different sensitivities to agonists and indeed may even influence the development of CHF itself.(Dishy, Sofowora et al. 2001; Small, Wagoner et al. 2002) One assessment of the MERIT-HF study shows that there is a clear disparity in the reaction of different individuals to beta-blocker doses: whilst some patients achieve a significant heart rate response with target doses, others may achieve a similar heart rate reduction with much smaller doses.(Wikstrand, Hjalmarson et al. 2002) The benefit in terms of clinical outcome seen was similar in both groups. Thus the effect of the beta-blocker on heart rate would seem to be more relevant to outcome than the dose of the beta-blocker used to achieve the heart rate

reduction. It may be simply that the sample of patients included in our study is not sufficiently representative of the population as a whole.

Whilst recognising this issue as a major practical difficulty in assessing the effects of the two drugs on the parameters studied, there are nonetheless differences apparent between the groups that cannot be readily explained by different beta-receptor polymorphism distributions. In particular, the finding a greater peripheral action of carvedilol, and of a difference in the antioxidant effects of metoprolol versus carvedilol is of interest.

Thus, in favour of the third possibility, it may be that the timing of measurements may have erroneously indicated a greater effect with metoprolol than with carvedilol. By measuring resting haemodynamic parameters and haemodynamic parameters influenced by the beta-agonist infusions a couple of hours after drug administration, it may be that the effect of metoprolol was actually exaggerated. Presumably the drug was exerting its peak effect at the measurement times. It is interesting to speculate that if this is indeed the case, it is likely that the effect of twice-a-day metoprolol is to produce alternating periods of excess and under-beta blockade. This would probably not be desirable, and may be another reason for the less favourable results for patients treated with metoprolol in COMET. What seems clear is that this formulation and dosing regime of metoprolol is not the equal of carvedilol given twice daily.

11.4. DIRECTIONS FOR FURTHER RESEARCH

The last few years have seen an increased understanding of the details of oxidative stress, and of the serious limitations in our understanding of this phenomenon, especially with regard to CHF.(Mak and Newton 2001) Future assessment of the antioxidant properties of carvedilol in CHF should employ more specific markers of oxidative stress.

Beta-blocker therapy has become a standard of care for patients with CHF. However, some aspects of therapy remain unclear. The dichotomy in response to metoprolol in MERIT-HF is one area of interest.(Wikstrand, Hjalmarson et al. 2002) It is also of note that there were apparent racial (and therefore presumably genetic) differences in response to bucindolol within the BEST study population.(BEST Trial Investigators 2001) This area is of particular importance if one were to undertake future small-scale studies of the effects of beta-blockers similar to that presented in this thesis. It would be important to know that the population under study would be uniform in distribution of any genetic variation. This may have been a factor causing the unexpected heart rate effect of metoprolol in this study.

This area of so-called “pharmacogenomics” takes as its premise that there are different degrees of response to pharmacological agents dependent on underlying genetic polymorphisms. That there are polymorphisms of β_1 receptors is well recognised,(Small, Wagoner et al. 2002) though the clinical relevance of this knowledge to the application of beta-blockers in CHF is unclear.(Hajjar and MacRae

2002) Further exploration of this area is surely one of the most fascinating current frontiers in medicine.

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